Plant Growth Regulation 19: 153–175, 1996.

Benzyladenine and derivatives – their significance and interconversion in plants

J. van Staden & N.R. Crouch
Natal University Research Unit for Plant Growth and Development, Department of Botany Pietermaritzburg, University of Natal, Private Bag X01, Scottsville 3209, South Africa

Received 6 September 1995; accepted 4 December 1995

Key words: benzyladenine, biochemistry, interconversion, metabolism, physiology

Abstract

Recently benzyladenine has been isolated as a natural cytokinin from a number of plants. The natural occurrence of this cytokinin will change the attitude with which physiologists view this hormone. This review attempts to put into context what is known about this cytokinin and its derivatives and to compare and contrast its metabolism and the function and physiological action of its various metabolites. Nothing is known about the biosynthesis of benzyladenine. Its structure would suggest that its biosynthetic pathway may differ considerably from that of zeatin and *iso*-pentenyladenine.

Abbreviations: Ade = adenine; Ado = adenosine; BA = benzyladenine; [9R]BA = BA ribonucleoside; [9R-MP]BA = BA nucleotide; [9R-DP]BA = BA dinucleotide; [9R-TP]BA = BA trinucleotide; [3G]BA = BA 3 glucoside; [7G]BA = BA 7 glucoside; [9G]BA = BA 9 glucoside; [9R-G]BA = BA 9-ribosylglucoside; [9Ala]BA = BA alanine-conjugate; (2OH)BA = BA ortho-OH; (2OH)[9R]BA = BA ortho-OH-riboside; KN = kinetin; [9R]KN = KN ribonucleoside; DHZ = dihydrozeatin; Z = *trans*-zeatin; [9R]Z = zeatin ribonucleoside; [7G]Z = zeatin-7-glucoside; [9G]Z = zeatin-9-glucoside; [9Ala]Z = zeatin alanine-conjugate; (OG)[9R]Z = O-glucoside of zeatin ribonucleoside; [9R-MP]Z = zeatin nucleotide; iP = *iso*-pentenyladenine; [9R]iP = iP ribonucleoside.

1. Introduction

Despite its recent identification as a naturally-occurring plant product [156] the purine cytokinin 6-(benzyl-amino)purine (Benzyladenine; BA) is still generally viewed as a synthetic compound. It is widely used in plant systems and frequently analogies are drawn between it and the synthetic, kinetin, 6-(furano-sylamino)purine and naturally occurring zeatin, (6-(4-hydrozy-3-methylbut-*trans*-2-enylamino) purine), with respect to synthesis, activity, metabolism and biological activity. This approach does not necessarily give a true picture of the role of cytokinins in general in plant growth and development. This review deals specifically with BA in an attempt to get an overview of what is known about the metabolism physiology and biochemistry of this cytokinin.

Although the biochemical and physiological effects of cytokinins are well documented [121] and structure-activity patterns have emerged [132, 226], their precise action remains unknown. One prerequisite for progress in the understanding of the molecular basis of cytokinin action would seem to be a detailed knowledge of cytokinin uptake and metabolism in plant cells [47]. 'Multilevels of experimental approach' have been advocated [32] for the elucidation of the mechanism of cytokinin action. Lack of determining the active form(s) of cytokinin is probably one of the most significant unsolved problems in cytokinin research. Currently it is not known if cytokinin activity *in vivo* occurs specifically at the level of the base, riboside, or ribotide [203]. Cytokinins may not be active as such, but only after metabolic transformation into other substances [72]. Such substances may not necessarily be recognisable as cytokinins. This may explain the

limited success of hormone receptor studies to date [23].

2. The free base-benzyladenine [BA]

Benzyladenine (BA) affects the growth of both animal [15] and plant [132] cells. The base BA is an adenine derivative with a substitution on the sixth position of the purine nucleus [191]. Recently, this cytokinin was found as a free, naturally occurring cytokinin [155]. This compound has been shown to affect plant metabolism and a wide variety of physiological responses have been recorded.

Besides delaying senescence [66, 78] including both floral [216] and monocarpic senescence [128], BA promotes chlorophyll retention [181] as well as its formation [44]. Thus cytokinin enhances photosynthetic activity [1, 28, 115] and reduces respiration rates [181]. The application of BA has resulted in increased shoot to root ratios [102], increased production of ethylene [181], lowered stomatal resistance [76], increased leaf expansion [187] and stimulated protein synthesis [199]. Adverse environmental conditions have been counteracted through use of BA, including heat stress [28]. It was not shown whether this was due to BA itself or to an increase in the natural cytokinin levels. A stimulative effect of BA on plant mineral nutrition was associated with an effect on the levels of endogenous cytokinins [102].

Applied as the base, BA is currently the most frequently and most successful cytokinin used in micropropagation [204]. However, when applied to field crops, BA showed disappointing results in delaying senescence [53]. Zhang et al. [241] suggested that the 'design' of cytokinins which are more field-effective than BA 'would be facilitated by a study of the metabolism of BA'.

Morris [151] suggested that kinetin applied to roots may be converted to an endogenous cytokinin before export to the shoot. However, within elm shoots, [8-^{14}C]BA appears to be largely transported in the unmetabolised state [17]. The cytokinin ribosides are generally considered the translocatory cytokinin species [94, 229]. Despite the report on BA transport in elms, research on the potential conversion of BA to its riboside [9R]BA or to endogenous cytokinin may prove profitable.

Much circumstantial evidence derived from bioassays exists to indicate that as a base, BA is the active cytokinin form [14]. Matsubara [132] considered BA to be the most active cytokinin in the class of ring-substituted aminopurines. Cytokinin-binding protein studies [34] have more directly implicated cytokinin bases as one of the active forms. Although the base is assumed to be active *per se*, there is no unequivocal evidence to support this proposition [222, 235]. To date, this issue has not been unequivocally resolved.

Laloue and Pethe [104] presented results on growth studies with tobacco cell cultures which indicated that conversion of cytokinin ribosides to bases is necessary for activity. Uptake of exogenously supplied BA by a variety of experimental systems was mostly linear in relation to the external BA concentration, suggesting a passive role [108, 143, 227]. However, uptake of cytokinin base has also been related to the rate of cytokinin metabolism inside the cell [45, 49].

In the soybean callus bioassay [141], BA gave an optimum response when applied at a concentration between 10^{-6} and 10^{-5} *M* [213]. Activity in a similar range has been recorded for zeatin [225]. Van Staden [209] compared the activities of BA, [9R]BA and [9R-MP]BA in the same bioassay system. Of these three, BA appeared most active, with the riboside more active than the nucleotide. It was suggested that the applied cytokinins might not have been taken up by the tissue at the same rate, or that differences in the metabolism of these metabolites occurred. In this case, BA may have been taken up quicker, or metabolised more slowly if active *per se*, or quickly converted to the 'active form' of cytokinin. Problems associated with the exogenous application of cytokinins to plant systems are many and varied.

Hecht et al. [84] reported that both the nucleoside and nucleotide were less active than the corresponding base. Their findings led to the suggestion that exogenous bases do not require activation before the expression of cytokinin activity. Similarly, Laloue et al. [107] reported that iP was three times as active as its nucleoside in a tobacco callus bioassay. However, Mok et al. [150] reported that reversed activities of cytokinin bases and nucleosides were detected with iP and [9R]iP in some *Phaseolus* callus cultures.

Peters and Beck [176] reasoned that cell division-controlling substances would be expected in highest concentrations during the logarithmic phase of cell growth. Yet, at the start of the log phase in *Chenopodium* cell suspensions, free bases were detected in low concentrations, suggesting that bases may not be involved in the regulation of cell division activity. However, other researchers [85] are of the opinion that low non-polar cytokinin levels do not neces-

sarily reflect cytokinin inactivity in tissue, but rather implies their active metabolism, coupled to cytokinin action. Future research which considers endogenous cytokinin levels before, during, and after physiological responses, may yet result in a re-evaluation of the concept of 'cytokinin activity' and the molecular species associated with it.

3. Benzyladenine riboside – [9R]BA

The 9-riboside of BA ([9R]BA) is a naturally-occurring cytokinin in anise plant cells [64]. Following exogenous application of BA, [9R]BA has been identified as a prominent [54] and sometimes dominant metabolite from a variety to species [80, 134, 236]. The analogous riboside of zeatin ([9R]Z) was the main detectable metabolite when zeatin was supplied to detached leaves of *Xanthium strumarium* [87]. In *Vinca rosea* crown gall tissue, [9R]Z and the corresponding O-glucoside ((OG[9R]Z) were found by mass spectrometric technique to be the most abundant natural cytokinins [185]. Other systems have been recorded as metabolising applied BA quite differently. Conversion of BA to [9R]BA was almost negligible in radish seedlings [235].

The 9-β-D-ribonucleosides of $\underline{N}^6$-adenine derivatives have been synthesised and tested [113, 114]. Their activity in the tobacco bioassay is not as high as the corresponding base. Metabolites of BA substituted at position 9 on the purine ring were less active than the base in the soybean bioassay [74]. These authors attributed this lower activity to the difficulty which the tissues may have in converting such compounds to the base. Riboside degradation through isoprenoid sidechain cleavage (resulting in adenosine formation) has been correlated with the weak activity of [9R]iP in some tissues (*P. vulgaris* cv. Great Northern) [148]. Tissues of *P. lunatus* cv. Kingston converted the unsaturated riboside to the corresponding nucleotide, and activity was maintained. This investigation highlighted the prominent intra-specific differences in cytokinin metabolism which occurs naturally.

The free base BA was among metabolites formed from the 9-substituted cytokinin, 6-benzylamino-9-methylpurine, suggesting that the biological activity of 9-substituted cytokinins could be accounted for by their conversion to the free base [73]. Other reports confirm the relatively low activity of cytokinin ribosides, both natural and synthetic, in a variety of bioassay systems [83, 107, 118, 133, 182, 196, 209, 233]. Generally, a second substituent in the 9-position of $\underline{N}^6$-substituted purines lowers, but does not eliminate cytokinin activity [132, 192].

Not all researchers consider the riboside less active than the corresponding base. Bopp and Erichsen [22] viewed observed differences as more a consequence of restricted uptake than of an inefficiency of the substance. Peters and Beck [176] have recently considered the endogenous cytokinin patterns at all growth stages of a *Chenopodium* cell culture. They considered that cytokinin ribosides likely control cell division, more so than free bases which have traditionally been considered the active form. It remains to be determined whether activity resides in the ribonucleosides, or is acquired only on conversion to their bases. Most evidence to date has supported the latter concept [73].

Cytokinin ribosides are generally considered to be the translocatory species [163]. Trans-membrane transport of the 9-riboside of kinetin ([9R]KN) was determined by Van Staden and Mooney [224], using *Catharanthus roseus* crown gall callus. Earlier, Laloue et al. [105] had shown ready uptake of [9R]BA by tobacco cells. Movement of cytokinins within the whole plant also occurs at the riboside level. Ribosides have been detected in the xylem sap of several species, including *Urtica* [229], *Phaseolus* [179] and radish [79]. After exogenous application of BA, [9R]BA was found in senescing *Xanthium pennsylvanicum* leaves as a major product [134]. Such evidence strengthens the view [94, 121] that the riboside is a translocatory form which is exported along with other important compounds from leaves prior to senescence. Following supply of ^{14}C-BA to *Phaseolus vulgaris* plant roots, only [9R]BA was detected in xylem sap collected from the stem [179]. Similarly, the riboside was the only significant source of radioactivity in the xylem sap of radish seedling after application of ^{14}C-Z [79]. These findings indicate that translocation of cytokinins from the roots is in the riboside form. Notably, ribosides are also major cytokinins in the phloem sap [121], indicating that the transport of ribosides may also be transported from shoots to roots.

A storage function for nucleosides has also been implied. A cytokinin riboside ([9R]Z) was detected in the (storage) roots of chicory by Bui-Dang-Ha and Nitsch [25]. It was not determined whether this riboside was synthesised *in situ* or merely stored there.

4. Benzyladenine-nucleotide-[9R-MP]BA

Following application of BA, [9R-MP]BA has been identified as a metabolite of BA in *Lemna minor* [16] soybean callus [70] and in *Acer pseudoplatanus* cell cultures [47]. The monophosphate of BA is relatively stable, as shown by its metabolic half-life in tobacco cells (8 days) [105]. In other systems which initially produced cytokinin mono-nucleotide as the principal metabolite, levels rapidly became subdominant [79].

Laloue and Pethe [104] considered cytokinin riboside-5′-phosphates to play a central role in the regulation of the levels of the various metabolic forms of cytokinins as they are readily interconverted to the riboside, and to the base. A role for cytokinin mono-nucleotides in hormonal homeostasis is generally accepted [164]. The main feature of inter-conversion pathways in this active cytokinin pool is that their overall equilibrium is thought to be in favour of nucleotide formation [105], although such conversion may involve a steady state maintenance of base and riboside levels [104]. Support of this homeostatic notion is provided by the fact that when cytokinin-dependent soybean callus was fed [9R-MP]Z [221], only the corresponding base and nucleoside were produced [104]. Laloue et al. [105] claimed that nucleotide isolation and identification has been neglected. These workers proposed that more attention should be paid to ribotides as naturally occurring cytokinins with a central role. A later report by Scott and Horgan [184] which employed mass spectrometric techniques has shown that cytokinin nucleotides may be more abundant than has been previously shown. These authors demonstrated that the nucleotide is more abundant than the ribosides in tissues where this was previously seen to be otherwise. Scott and Horgan [184] predicted that the application of 'new analytical techniques for cytokinin nucleotides will result in an extensive re-evaluation of the existing cytokinin literature'. Such a re-evaluation has not yet occurred.

According to Ashihara [8], purine nucleotidase are synthesised both from i.e. the *de novo* pathway amino acids, CO_2, tetrafolate derivatives and α-5-phosphoribosyl-1-pyrophosphate (PRPP) and from preformed purine bases and their ribonucleosides (the salvage pathway). Nothing is known of BA biosynthesis in plants [99]. Should biosynthesis of 6-(benzylamino)purine proceed at the nucleotide level, as suggested for *iso*-pentenyl-type cytokinins [121, 201], then [9R-MP]BA will likely play an essential role as an intermediate precursor in those tissues where naturally-occurring BA metabolites are known to occur [197]. However, in this cytokinin there is a benzyl ring attached at the ^{6}N-position of adenine. This makes it unlikely that iso-pentenyl transferase would be involved. It seems that a common biosynthetic pathway may not exist for iP and BA cytokinins.

Once in the active pool, the nucleotide may exist *via* the base or the riboside to N-conjugates, or oxidative catabolites. [9R-MP]BA has been implicated as the immediate precursor of [7G]BA in tobacco systems [71]. Conversely, [7G]BA applied to tobacco was (indirectly) converted to BA nucleotides [77]. These authors suggested that such a conversion is indirect, via the transient formation of BA. As with the other cytokinin species which contribute to the active pool, the exact role(s) of nucleotides remains to be fully elucidated.

Nucleotides have been associated with storage of cytokinins [121, 209]. Pietraface and Blaydes [177] provided evidence to show that the nucleotide is a storage from in lettuce seeds before conversion to the active nucleoside.

Nucleotide formation may also be associated with cytokinin uptake [164] and transport across membranes, in much the same way as phosphoribosylation plays a role in the uptake of adenine by *Escherichia coli* membranes [121]. Burch and Stuchbury [27] noted that although polar cytokinins such as nucleotides are common metabolites within cells, they have rarely been identified in the culture media. This has been considered indicative of plasmalemma impermeability to [9R-MP]BA [104, 105]. However, following incubation of [9R-MP]Z with soybean callus, various zeatin metabolites were extracted from cellular contents. High levels of [9R-MP]Z detected in artichoke tissues shortly after the start of culture was cited as evidence [164] that the nucleotides are involved in cytokinin uptake. Yet, with the aid of adenine phosphoribosyltransferase-deficient (APRT) mutants, Moffatt et al. [145] have recently shown that phosphoribosylation of BA is not a prerequisite for its uptake by *Arabidopsis* plants.

A translocatory role for this cytokinin class has been proposed. Free base applied to bean roots was recovered in the stem, partly as the nucleotide [232]. Both ribosides and ribotides were identified by Palmer et al. [163] in stems of decapitated, disbudded bean plants. Vonk and Davelaar [228] suggested that the nucleotides are cytokinins in transport in the phloem. The highest levels of [9R-MP]BA detected in tomato

plants were in the stems [14], again implying a translocatory role.

Shaw et al. [189] considered the cytokinin activity of the 3-, 7-, and 9-methyl derivatives of zeatin. The results implied that the mechanism of cytokinin activity in substituted adenines does not require prior formation of nucleotide derivatives. In contrast, Laloue et al. [105] correlated division of cytokinin-requiring tobacco cells with high levels of cytokinin ribotides and low levels of cytokinin base and riboside, irrespective of whether the base or riboside was supplied. The fact that the methyl group in the 9-position does not constitute an effective block, but is easily metabolised, makes suspect the presumed stability of other 9-substituted cytokinins [73]. When compared in the tobacco callus bioassay, [9R]BA and [9R-MP]BA were less active at lower concentrations (10^{-1} μM) than BA [183]. More recently, similar results were obtained using the soybean callus bioassay [219].

The identity of the actual 'active cytokinin' form(s) remains an unresolved issue. The nucleotide may be necessary for the expression of activity [105], but if not, is likely to contribute to the steady state maintenance of an active cytokinin pool.

5. Di- and tri-phosphates of benzyladenine ([9R-DP]BA and [9R-TP]BA)

The di- and tri-nucleotides of BA have been detected in extracts of *Petunia* leaves following incubation with BA [10, 11, 12]. These metabolites were rapidly produced by the explants and were considered by these authors to be active forms responsible for shoot induction. An earlier indication of the activity of cytokinin polynucleotides was revealed by Miller [140]. This author extracted a cytokinin from *Zea mays* kernels possessing 'at least two phosphate groups', which showed some activity in a soybean callus bioassay. Bezemer-Sybrandy and Veldstra [16] detected mono-, di, and tri-nucleotides of BA as metabolites in *Lemna minor* cultures. The formation of such nucleotides was considered to be a normal feature of cytokinin metabolism in plant tissues [140, 206], and to indicate the natural occurrence of analogous endogenous nucleotides in plant tissues [106].

The existence *in vivo* of cytokinin nucleoside-5′-triphosphate is of theoretical importance as such compounds could be incorporated into RNA molecules [69, 106] to provide a basis for cytokinin action. Incorporation of BA into RNA was demonstrated by Fox [68]. Armstrong et al. [5] similarly viewed [9R-TP]BA as an important intermediate in a pathway for the incorporation of BA into RNA species. The incorporation of cytokinin bases into polynucleotides [33] indicates that the 5′-monophosphate is an intermediate metabolite in the reaction. Preferential incorporation of label into the guanine fraction of soluble RNA hydrolysates from soybean and tobacco callus cultured on media containing ^{14}C-BA was recorded by Fox [69]. In soybean callus, [9R-MP]BA appeared as the major metabolite [70]. Cytokinin-dependent tobacco callus supplied with BA incorporated this compound in low levels in both tRNA and rRNA though mainly in the rRNA [5]. Jouanneau and Teyssendier de la Serve [98] considered this to occur through a direct insertion process. Despite the large number of BA metabolic studies in plant tissues, detection of di- and tri-nucleotides has rarely been reported. When identified, these compounds are normally minor metabolites [106]. *Acer* cultures supplied with N^6-substituted nucleosides did not phosphorylate these compounds beyond the monophosphate level [47, 48]. In contrast, 3 hours after application of ^{14}C-BA to tobacco cell cultures, 6% of the radioactivity was associated with [9R-DP]BA and [9R-TP]BA [106]. The monophosphate ([9R-MP]BA) represented 28%, the base 30% and [9R]BA was unrepresented. To explain differences in the metabolism of BA observed between *Acer* and tobacco, Laloue et al. [106] suggested that the cytokinin inactivation through sidechain cleavage noted for *Acer* [47] would restrict formation of the di- and tri-nucleotides.

The hydrolytic action of 5′-nucleotidases which show equal affinity for mono-, di- and tri-phosphates of adenosine [35] are likely responsible for release of [9R]BA from [9R-DP]BA and [9R-TP]BA. Such a conversion could produce a more active cytokinin species, either in the form of [9R]BA, or after deribosylation of [9R]BA to the base.

6. N-conjugation of BA

Collectively, the N-alanyl conjugates and N-glucosides of cytokinins are referred to as N-conjugates.

N-conjugates are stable both when applied externally and when found as metabolites [170, 173] and are generally incapable of further metabolism back to base [136, 138]. For this reason N-conjugates are regarded as detoxification or inactivation products [77, 121] rather than storage forms; a role proposed for the

O-glucosides of zeatin [75]. Hence N-conjugation may result in the irreversible loss of cytokinin activity.

In reducing levels of cytokinin activity, plants may oxidise the 'active compound' [198], or alternatively glucosylate/alanylate. McGaw and Horgan [138] distinguished an 'oxidative-type' metabolism from a 'glucosidase-type' metabolism to describe either oxidative cleavage of the N^6-sidechain, or conjugation of exogenously supplied cytokinin. In many tissues BA is resistant to attack by cytokinin oxidase. Consequently, N-conjugation may provide the only mechanism by which the biological activity of this cytokinin might be controlled. Side-chain cleavage of N-conjugates [168] may be a means of reducing still further the activity of these detoxification products.

7. Benzyladenine - Metabolism

Although application of a cytokinin metabolite to a bioassay system may promote an active response, each of these 'active' compounds are themselves rapidly metabolised to an extensive range of products, many of which are active to varying degrees in the same bioassay [219]. Mok et al. [146] observed that the activity of a particular cytokinin may depend on the bioassay system used. Activity differences between metabolites were attributed by these authors to reflect uptake, compartmentation, sensitivities to enzymes, or binding site specificities. In different bioassays these components may change, so altering the effectiveness of particular metabolites in inducing a response. In an alternative experimental approach, studies of endogenous cytokinin levels during different phases of plant growth [176] have provided insight into *in vivo* biological activity. the manipulation of endogenous cytokinin levels by genetic transformation has also been considered more useful in revealing natural processes than exogenous applications to isolated organs or calli [3, 82].

Plant tissues convert exogenous BA into a great diversity of metabolites which include products of ring substitution (ribosides, nucleotides, N-glucosides), and products of sidechain cleavage (e.g. adenine, adenosine, and adenosine-5′-monophosphate [121].

The functional significance of these metabolites remains obscure [229], but it has been suggested [121] that these compounds could be:

1. Active forms of cytokinin, i.e. the molecular species which bind to a receptor to evoke a growth or physiological response;
2. Translocation forms;
3. Storage forms which would release free (active) cytokinin when required;
4. Detoxification products formed following exogenous cytokinin application at toxic levels;
5. Deactivation products formed to lower endogenous (active) cytokinin levels; and
6. Postactivation products, formation of which is coupled with cytokinin action (formed as a result of cytokinin utilisation).

McGaw and Horgan [138] indicated that an understanding of compartmentation with respect to the mechanisms and sites of cytokinin action needs to occur before the exact roles of various cytokinins may be assigned. Until this knowledge is obtained and activities can be measured directly at the site of action [146] prescribed roles will remain mainly speculative.

Chen [32] considered fundamental control mechanisms to be those operating at the level of enzymic regulation of metabolism (biosynthesis, interconversion, and degradation). Several major enzymic pathways compete for cytokinins, by which they are inter-converted and degraded [32]. Burch and Stuchbury [27] noted that enzymes metabolising adenine derivatives [34, 35, 38] exhibit a low degree of specificity for the exact structure of the purine ring and hence the same enzymes will actively metabolise many N^6-substituted cytokinins albeit at different rates. The fate of a cytokinin may be attributed to the relative activities of cytokinin metabolic enzymes, which in turn are affected by the relative concentrations and distribution of the hormone and its precursors in the plant cell [32]. Given the lack of specificity of some cytokinin-metabolising enzymes, metabolism of cytokinins may be limited by competition for the enzymes. Hence Burch and Stuchbury [27] stated that 'interpretation of many aspect of cytokinin biochemistry is dependent on a much better understanding of the relationship of their metabolism to that of other purines'. Much is known of cytokinin metabolism, but no common metabolic pattern has emerged. Several factors may have contributed to this complexity. The stage of plant development [27, 43, 119], physiological condition [75, 162], organ type [14, 27], plant species used [20], concentration of supplied compounds [213], and method of application [223] have all been shown to have an effect on the metabolism of exogenous and endogenous cytokinins.

7.1 *Inter-conversion within the active cytokinin pool*

The free base, nucleoside and mononucleotide forms of cytokinins all appear to be readily inter-convertible in plant tissues [121, 215]. These cytokinin species are considered the functional forms [126]. The early formation of the 9-riboside ([9R]BA) and 9-ribotide of BA ([9R-MP]BA) by many systems as the principal metabolites of BA [12, 72, 126, 158, 220, 223] could be a mechanism for maintaining an active cytokinin pool. This would ensure a continued supply of precursors for subsequent conversion to the base (or active form). Di- and tri-nucleotides of cytokinins do not appear to contribute significantly to this active pool [106].

Should the biosynthetic pathway for BA, like that for 6-)3-methylbut-2-enylamino)purine (iP) proceed at the nucleotide level [201] then subsequent conversion to base and riboside would be expected. Cytokinin bases can be continuously catabolised by various enzymes to form adenine and other degradation compounds [67, 134], resulting in a loss of cytokinin base. Such a loss may need to be replenished in order to maintain the levels of available cytokinin (the so called 'active form') [34]. De-glucosylation [65], de-alanylation [239], deribosylation [34], and de-phosphoribosylation [221] would provide the needed cytokinin base.

The enzyme catalysing inter-conversions within the active pool are likely not cytokinin-specific, but rather those which catalyse analogous reactions for adenine, adenosine, and AMP [121, 145]. Other workers [221] have viewed such enzymes as cytokinin-specific.

Burch and Stuchbury [27] listed a series of reactions and the enzymes responsible for their inter-conversion (Table 1). The analogous conversions of the cytokinin 6-(benzylamino)purine are also shown.

Two mechanisms for the incorporation of purines into nucleotides have been proposed:

1. A two-step process involving first *nucleoside phosphorylase* to yield a cytokinin riboside and then *adenosine kinase* to catalyse nucleotide formation.
2. A one-step process involving a direct transfer of a ribose-5^1-monophosphate group from α-5-phosphoribosyl-1-pyrophosphate (PRPP) to the base, catalysed by an *adenine phosphoribosyltransferase*.

A one-step phosphoribosylation is not universally accepted. Evidence exists to support both the one-step [47, 104] and two-step [216] pathways. The two-step route may be limited by the restricted occurrence of *nucleoside phosphorylase*, rather than by *adenosine kinase* activity, which appears ubiquitous in plants [48].

7.2 *Conversion of benzyladenine to its riboside (BA — [9R]BA)*

Ribonucleosides have been shown to be formed in plant tissues when the corresponding base was supplied [195]. The significance of *adenosine phosphorylase* activity in purine salvage reactions has been the subject of considerable debate. Phosphoribosylation catalysed in a single step by adenine phosphoribosyltransferase (APRT) is viewed as the main pathway [109]. However, in APRT-lacking mutants of *Arabidopsis*, limited formation of [9R-MP]BA revealed some activity of the two-step reaction involving adenosine phosphorylase [145].

Although detected in bacterial systems [90, 188], the occurrence of adenosine phosphorylase in plants was initially questioned [48], and is still viewed by some researchers [27] as limited in distribution. This enzyme was purified from wheat germ cells by Chen and Petschow [39]. Conversion of the base to the riboside requires the addition of ribose-1-phosphate. In the presence of inorganic phosphate, phosphorolysis of nucleosides occurs [32, 188]. However, Chen and Petschow [39] noted that the equilibrium constants for the phosphorolysis of [9R]iP and iP indicate that nucleoside formation is in the favoured reaction. Chen [32] suggested that purine nucleoside was the enzyme catalysing cytokinin nucleoside formation, as distinct from purine nucleoside phosphorylase which is generally considered to be inactive towards Ade, Ado, and cytokinin nucleosides [242]. Senesi et al. [188] were the first to clearly distinguish adenosine phosphorylase from purine nucleoside phosphorylase. Adenosine was not a substrate for purine nucleoside phosphorylase, unlike the nucleosides of hypoxanthine and guanine.

7.3 *Conversion of BA riboside to its base ([9R]BA — BA)*

Formation of the base may involve a deribosylation of the free riboside [105, 208] or may be the result of a direct (reversible) dephosphoribosylation of the nucleotide [139]. Cytokinin base has been reported as a metabolite formed from the nucleoside [159, 215], and may represent an activation step [180].

The products of the hydrolytic nucleosidase from *Lactobacillus pentosus* was shown [231] to be the purine, and free ribose. The enzyme was not expected

Table 1. Inter-conversion within the active cytokinin pool, catalysed by non-specific enzymes of adenine metabolism

Enzyme	Class	Reaction catalysed for Ade	Analogous BA conversion
5^1-nucleotidase	EC 3.1.3.5	AMP + H_2O — Ado + Pi	[9R-MP]BA + H_2O — [9R]BA + Pi
Adenosine nucleotidase	EC 3.2.2.7	Ado + H_2O — Ade + ribose	[9R]BA + H_2O — BA + ribose
Adenine phosphoribosyl transferase	EC 2.4.2.7	Ade + PRPP — AMP + PPi	BA + PRPP — [9R-MP]BA + PPi
Adenosine phosphorylase	EC 2.4.2.1	Ade + R-1-P — Ado + Pi	BA + R-1-P — [9R]BA + Pi
Adenosine kinase	EC 2.7.1.20	Ado + ATP — AMP + ADP	[9R]BA + ATP — [9R-MP]BA + ADP

Pi – inorganic phosphate; PPi – inorganic diphosphate; R-1-P – ribose-1-phosphate; PRPP – α-5-phosphoribosyl-1-pyrophosphate; Ade – Adenine; Adenosine – Adenosine.

to exist because of the wide distribution of the phosphorolytic nucleosidase in animal tissues and in micro-organisms. Whitty and Hall [234] termed this enzyme isolated by Wang [231] 'purine nucleoside hydrolase'.

Adenosine nucleosidase catalyses the irreversible deribosylation of Ado, to give Ade and ribose [26]. Three separate adenosine nucleosidase enzymes were partially purified from tomato roots and leaves [26]. These workers found the conversion of Ado to Ade to be inhibited by the presence of [9R]BA, with substantial differences in the pattern of inhibition evidenced for each of the three enzymes. Earlier, Chism et al. [40] distinguished between cytokinin nucleosidases and adenosine nucleosidases in tomato fruits. When the $\underline{N}^6$-amino group of Ado was replaced by an isopentenyl amino sidechain in substrates of adenosine nucleosidase, the K_m value of the reaction was decreased by a factor of 1.7 [34]. The cytokinin base, BA, appeared a suitable substrate in this reaction, as the adenosine nucleosidase exhibited a specificity for Ado and $\underline{N}^6$-derivatives of Ado. From wheat germ cells, a partially purified adenosine nucleosidase (EC 3.2.2.7) catalysed the irreversible hydrolysis of the riboside of iP ([9R]iP) to iP, and adenosine to adenine [32, 39]. The activity of such adenosine nucleosidases appears to depend on the plant tissue investigated [159, 203]. Significant differences in adenosine nucleosidase activity were detected between wild-type and domesticated plant species [116].

Adenosine nucleosidase activity has also been detected in soybean [142], beet [172], and barley leaves [81].

7.4 *Conversion of benzyladenine riboside to its nucleotide ([1R]BA — [9R-MP]BA)*

Enzymic preparation of mono-nucleotides from $\underline{N}^6$-substituted adenosines and ATP is catalysed by adenosine kinase (EC 2.7.1.20). Such activity has been found in both yeasts and higher plants [48]. Adenosine kinase activity was demonstrated in buds of *Cicer arietinum* and in suspension-cultured cells of *Acer pseudoplatanus*.

Time course studies with *Phaseolus vulgaris* [179] and *Dianthus caryophyllus* [216], have indicated formation of cytokinin nucleotides from the corresponding nucleoside. More direct evidence was provided by Chen and Eckert [33] who reported that cytokinin nucleoside could be converted to the nucleotide (5^1-monophosphate) by adenosine kinase isolated from wheat germ cells. The phosphorylation of [9R]iP depended upon the presence of ATP and Mg^{2+}. The enzyme activity responsible for such synthesis was considered by Doree and Terrine [48] to be ubiquitous in plants.

7.5 *Conversion of benzyladenine nucleotide to its riboside ([9R-MP]BA — [9R]BA)*

Following application to soybean callus, [9R-MP]Z was rapidly metabolised to both the riboside and the base [221]. Ribonucleosides can be formed from the corresponding ribonucleotide or from the cytokinin base [32, 191]. Should the one-step pathway of nucleotide synthesis be dominant, then conversion of applied cytokinin base to the riboside may proceed through phosphoribosylation of the base to the nucleotide, followed by conversion to the riboside [39].

Conversion of cytokinin ribonucleotide to its nucleoside may be catalysed by 5^1-nucleotidase [35]. Such conversions have been indicated by time-course studies [130] and demonstrated during *in vitro* investigations [32]. This cytosolic enzyme consists of at least two forms, referred to as the F-1 and F-2 5'-nucleotidases, which specifically hydrolyse purine ribonucleoside-5^1-phosphates [32]. Notably, 5^1-nucleotidases have been reported [35] to show

almost equal affinity toward the mono-, di- and tri-phosphates of adenosine (AMP, ADP and ATP). The extent of dephosphorylation of cytokinin ribonucleotide in plant cells by acid phosphatase and membrane-bound 5^1-nucleotidase remains to be investigated [35].

7.6 *Conversion of benzyladenine to its nucleotide (BA — [9R-MP]BA)*

Quick metabolism of base to nucleotide has been demonstrated for both lower [63] and higher plants [79, 139]. A one-step purine salvage reaction catalysed by adenine phosphoribosyltransferase (APRT) is seen as the predominant pathway in plants [139, 145], as this enzyme activity is high enough to account for the salvage of Ade into AMP. Further, some researchers [109] maintain, despite the work of Chen and Petschow [39], that the presence of adenosine phosphorylase in plants has not been unequivocally demonstrated. From a time-course study on *Acer pseudoplatanus* cell culture, Doree and Guern [47] provided evidence to show that the synthesis of $\underline{N}^6$-substituted nucleotides does not proceed through a two-step reaction, but rather through the direct transfer of ribose-5^1-monophosphate. 6-(Benzylamino)purine was a suitable substrate.

Extracts from soybean (cv. Acme) similarly yielded APRT activity, as did senescing barley leaves [157]. This enzyme from soybean was inhibited by AMP (product feedback inhibition), and stimulated by ATP. The monophosphate of BA ([9R-MP]BA) was also found to inhibit AMP production, though not to the same extent (13 vs. 92%). Soybean callus contacting kinetin in the agar medium was shown to have increased APRT activity [157].

Adenine phosphoribosyltransferase has been partially purified from tobacco pith tissue cultures [33], and from wheat cells [32]. This enzyme was also extracted and partially purified from Jerusalem artichoke shoots [111, 112]. Phosphate ions and thiol-reducing substances were required to stabilise it.

However, Chen [32] showed that in wheat germ cells, cytokinin nucleotide is not preferentially formed by this one-step pathway as iP has a high K_m value. Krentisky et al. [101] had earlier reported that the enzyme binds to adenine through the 6-amino group and the 3- and 7-nitrogens. Chen [32] was thus not surprised to find iP (an adenine analogue with a modified 6-amino group) to show reduced ability as a substrate. It was suggested that a different form of this enzyme may exist for cytokinin bases in wheat. A later investigation revealed that APRT from the cytosol of wheat germ was capable of phosphoribosylating BA [38]. However, the ratio of $V:K_m$ indicated that adenine is approximately two-fold more efficient than BA as a substrate. Lee and Moffatt [109] have more recently purified and characterised an APRT from *Arabidopsis thaliana* which catalysed phosphoribosylation of BA. However, it was again not possible to fully resolve the physiological role of APRT with respect to BA.

In summary, the base, riboside and nucleotides of cytokinins appear to be readily inter-convertible within plant tissues. Enzymes responsible for catalysing these reactions are not likely to be cytokinin-specific, although in some tissues specific enzymes may be present [40]. The extent to which one-step or two-step phosphoribosylation of cytokinin bases occurs appears to be a function of the plant system investigated.

8. Benzyladenine glucosides

The metabolites of BA include a group of N-glucosides in which the sugar moiety is linked to a purine ring nitrogen atom. These are [3G]BA, [7G]BA and [9A]BA, of which the 3- and 7-glucosides are considered to be particularly unusual [12]. Transglycosylation reactions in which glucose is transferred enzymically from a glucoside to the free base BA to yield a different glucoside have not yet been reported [119].

Cytokinin O-glucosides, where glucose is substituted in the $\underline{N}^6$-sidechain of a molecule such as (20H) BA, have not been observed for BA, as they have for zeatin [91]. The O-glucosidic linkage in such compounds as (OG)Z and (OG)DHZ confers a greater liability to acid and β-glucosidase hydrolysis [166, 212, 219, 225] than has been observed with any N-glucosidic bonds. As a result, O-glucosides probably serve as translocatory [210, 217] and storage forms [75, 211], unlike the more stable N-glucosides. As the presumed roles of these two glucoside types fundamentally differ, analogies which may be made are limited. Accordingly, cytokinin-O-glucosides are not currently reviewed. However, it is noteworthy that not only glucose, but xylose has also been identified (from *Phaseolus vulgaris* embryos) as a cytokinin conjugate ((OX)Z) [147, 149]. Turner et al. [205] isolated and partially characterised the enzyme (UDP xylose: zeatin-xylosyl transferase) catalysing such conjugation, and showed its specific requirements for UDP-

xylose. To date, cytokinin-O-xylosides have only been detected in members of the family Leguminosae. Should the natural occurrence of an O-glucoside of (20H)BA or (20H)[9R]BA be indicated, then the existence of analogous O-xyloside of BA in selected plant species is conceivable. In this regard, the finding of (20H)[9R]BA in *Populus* × *robusta* [94] and the recent identification of (20H)BA as a natural cytokinin [197] makes the hypothetical existence of (OG)BA and (OX)BA more feasible.

Both [7G]BA and [9G]BA have been confirmed by synthesis [41], to be β-D-glucopyranosides. Parker et al. [175] considered the identification of the glucosides of zeatin and BA to be the first unequivocal evidence for the occurrence of purine glucosides in living tissues. Letham et al. [124] later demonstrated that glucosylation of such purines is not restricted to only N^6-substituted adenines with strong cytokinin activity.

Cytokinin N-glucosides have not been detected in xylem sap, and hence are apparently not supplied to the leaf from the root [50]. These N-glucosides are much less active in bioassays than the O-glucoside or the parent molecule [88, 118, 220]. They have alternatively been described as having 'enhanced metabolic stability' [96, 126, 173]. If these metabolites are the functional form of BA, then they would probably exhibit high cytokinin activity. The stability of N-glucoside metabolites is possibly due to their resistance to degradative enzymes [127] or to their compartmentation [126].

Letham et al. [126] found that formation of the 3-, 7- and 9- glucosides of BA was not dependent on BA concentration, in which case formation of the metabolites may not simply be a mechanism for inactivating physiological excesses of BA. Similarly, the rate of BA glycosylation in radish cotyledons [126] and tobacco cells [77] was found to be relatively insensitive to large differences in the concentration of supplied BA [165].

Entsch and Letham [58] claimed that the physiological significance of the 7- and 9-glucosides of cytokinins is uncertain, although it has been suggested [170] that they are storage forms of the hormone rather than the product of a detoxification pathway [77]. Entsch et al. [62] proposed that cytokinin glucosides may simply be waste-products formed by glucose transferases, which catalyse the formation of glucoside metabolites characteristic of a particular species. In summary, the significance of cytokinin metabolite formation, in particular glucosylation, has been variously associated with a detoxification mechanism, a method of storage, and a mechanism for lowering endogenous cytokinin levels [193].

8.1 *The 3-glucoside of bezyladenine – [3G]BA*

When supplied to de-rooted radish seedlings, BA was principally converted to 7-, and 9-glucosides. A third minor metabolite exhibited cytokinin-like activity markedly greater than that of these glucosides [235]. It was identified [127] as the first compound with a glycosidic linkage at position 3 of a purine ring to be isolated from a plant tissue. This compound was 6-benzylamino-3-β-D-glucopyranosylpurine ([3G]BA).

The 3-glucosides have not been isolated as endogenous cytokinins from any source, although [3G]DHZ appeared as a minor metabolite when DHZ was exogenously applied to de-rooted radish seedlings [136].

Letham et al. [122] considered a number of cytokinin bioassays and compared the activities of 3-, 7- and 9-glucosides of BA. Cytokinin activity was markedly reduced by 7- and 9-glucosylation in nearly all bioassays, but 3-glucosylation of BA had little effect on activity. The 3-glucoside of BA, produced as a minor metabolite of BA in *Dianthus caryophyllus* flowers, showed higher senescence-delaying activity than either the 7- or 9-glucosides [216]. Since 3-alkyl derivatives of BA are essentially inactive [192], the high activity of [3G]BA in diverse bioassays [122, 174, 220] is probably due to cleavage of the 3-glucoside moiety to release free BA. Such cleavage has been demonstrated in radish cotyledons [119, 126] and soybean callus [220]. The 3-glucoside of BA supplied to cytokinin-dependent soybean callus was rapidly metabolised to mainly BA, and another unidentified bioactive compound [220]. Release of appreciable amounts of BA from [3G]BA was considered by Letham and Gollnow [119] to account for the high activity of this glucoside in cytokinin bioassays. The 3-glucoside has been shown susceptible to hydrolysis by almond β-glucosidase [127, 174, 220]. The 3-glucoside is hydrolysed slowly by this enzyme whereas the 7- and 9-glycosyl metabolites of BA are not hydrolysed at a detectable rate by either α- or β-glucosidase [174]. Of the three N-glucosides of BA, [3G]BA was the most readily hydrolysed by acid [220]. These authors proposed that if steric factors cannot adequately explain the greater lability of the N-C glucosidic bond of [3G]BA in the presence of β-glucosidase, then compartmentation of the various glucosides may differ. The enzyme(s) responsible for [3G]BA formation have not yet been characterised.

Although more active than [7G]BA and [9G]BA as a cytokinin, [3G]BA is still only weakly active relative to the corresponding base when applied at physiological levels in bioassays [220].

8.2 *The 7-glucoside of benzyladenine – [7G]BA*

Raphanatin ([7G]Z) was the first purine glucoside to be identified [170]. It was earlier isolated from radish cotyledons by Parker et al. [172] who reported on its activity in the radish cotyledon bioassay. Prior to the report by Parker and Letham [170], Deleuze et al. [46] had isolated the 7-glucoside of BA from sliced potato tubers and based on spectral evidence, proposed a glucofuranosyl structure. The glucose ring size and stereochemistry of the sugar linkage of [7G]BA was later investigated [51] and found to be a 7-β-glucopyranoside. Similarly, although Fox et al. [71] reported on the existence of the 7-glucofuranoside of BA in potato tuber tissue, this was later shown [120] to be the pyranoside. The 7-glucoside of BA ([7G]BA) has been shown to be the major metabolite in tobacco, another solanaceous species [77, 207].

The base appears to be the precursor for 7-glucosylation, as expected from the consideration of the 7- and 9-tautomeric positions of the purine ring, although kinetic studies indicated that the ribonucleotide is the immediate precursor [71].

The roles of cytokinin-7-glucosides in controlling hormone activity remain unclear. the 7-glucosides of cytokinins are metabolically stable [77, 170], and weakly active relative to the unsubstituted cytokinin [103, 235]. McGaw and Horgan [138] considered [7G]BA as a deactivation or detoxified cytokinin form, which was biologically inactive. Laloue et al. [105] considered 7-glucosylation of BA as a (terminal) inactivation step as its formation was 'practically irreversible' and the rate of reutilization extremely slow [77]. When the amount of [7G]BA present in tobacco cells did not increase proportionately following further addition of BA, Gawer et al. [77] reasoned that 7-glucosylation formation may not be a detoxification step.

In contrast to the proposed terminal inactivity of [7G]BA, several researchers have viewed the 7-glucosides as storage forms of cytokinins [103, 104, 119, 170]. Laloue [103] suggested that they are storage forms as they are stable with respect to degradation that occurs upon N^6-sidechain removal [105] and because they can be converted to cytokinin nucleotides [77]. These authors suggested that this conversion is indirect. via the transient formation of BA. As β-glucosidases do not substantially. hydrolyse [7G]BA, then the existence of an enzyme which removes the glycosyl moiety at position 7 of the purine ring and simultaneously attaches a phosphoribosyl group should be considered.

Letham and Gollnow [119] suggested that the cytokinin-7-glucoside of zeatin may be a translocation and a storage form, given its resistance to degradation, its production at sub-optimal levels (hence not a detoxification form), and movement in radish seedlings [126].

Laloue [103] considered the effect of [7G]BA on cell division in suspension cultures of *Nicotiana tabacum*. He found that cytokinin-7-glucosylation was not involved in the expression of the biological activity of cytokinins. This report conflicted with the view of Fox et al. [71] who considered cytokinin-7-glucosides to be the 'active forms', as this metabolite was the only cytokinin species containing the intact cytokinin moiety that remained in actively growing cytokinin-requiring tobacco tissue in the long term. These authors had reasoned that as [7G]BA was not degraded through sidechain cleavage, the 7-glucoside may thus be the active form of the cytokinin. Additionally, substantial growth was detected in the soybean callus bioassay.

Soybean callus degraded [7G]Z to [7G]Adenine [168], showing that oxidation of [7G]BA may in fact occur. McGaw and Horgan [138] found that [7G]Z was metabolised to adenine, adenosine and [7G]adenine within two days. By inference, one would expect [7G]BA to be susceptible to β-glucosidase, although this has not yet been demonstrated. Van Staden and Drewes [220] later showed very little degradation by this enzyme, much less than for the more labile [3G]BA.

The lack of agreement on the biological significance of cytokinin-7-glucosides highlights the confusion surrounding most cytokinin metabolite roles. A lack of uniformity with respect to systems investigated and particularly bioassays employed, has probably been a major cause for such apparent contradictions. However, when one considers the wide array of bio-responses elicited by cytokinins, the varied experimental approaches are placed in context.

In summary, although early evidence permitted the interpretation of [7G]BA as an active or storage form of cytokinin, this compound is currently widely viewed as an inactive product of deactivation or detoxification mechanisms. This glucoside may be degraded further to oxidation products.

8.3 *The 9-glucoside of benzyladenine – [9G]BA*

Letham et al. [120] demonstrated the production of [9G]BA by potato tuber tissue. A considerable amount of this compound was found by Zhang et al. [241] to be produced in soybean leaves. The 9-glucoside of BA is a stable metabolite in radish cotyledons [235]. This glucoside was detected by Van Staden et al. [216] as a major metabolite in cut carnation flowers. Cytokinin base (BA) supplied to the stems was later recovered partly as [9G]BA in the stem, petals and receptacle.

The partially characterised cytokinin extract from rice roots and presumed to be a 9-glucoside [237] is more likely O-glucosylzeatin [91, 198, 230]. Following this report, Letham [117] identified a 9-glycoside of zeatin from corn kernels, although the identity of the sugar moiety was not established. Subsequently, the 9-glucoside of zeatin was isolated as a major metabolite from roots to *Zea mays* [171].

Oil palm *Elaeis guineensis* callus supplied with kinetin produced [9G]KIN as the major metabolite [97]. As oil palm cultures do not require added cytokinins, these authors suggested inactivation of an excessive cytokinin load had occurred. Jones and Hanke [97] noted that in cytokinin-autonomous *Elaeis* cultures, added cytokinins have not been shown to improve callus growth. Cytokinin-9-glucosides may then be formed in an inactivation or detoxification pathway. Earlier reports [170] have considered [9G]BA to be a storage form.

The stability of [9G]BA has been demonstrated: [9G]BA is resistant to enzymic degradation by *Escherichia coli* nucleoside phosphorylase and a nucleoside hydrolase from *Cicer arietinum*, unlike [9R]BA which was susceptible [80]. Van Staden and Drewes [220] similarly found [9G]BA to be a stable, inactive metabolite in soybean callus. When Guern [80] and co-workers were experimenting with [9G]BA, no purine glucosides were known to occur naturally. Recently, [9G]BA has been identified as a naturally occurring cytokinin [155].

When [9G]BA was injected into *Cicer arietinum* seedlings, it was apparently readily translocated without appreciable enzymic modification [80]. However, cytokinin-9-glucosides have not been detected as endogenous translocatory forms.

As [9G]BA is weakly active relative to BA [122, 219, 220, 235] specific inhibitors of the glucosylating enzymes may constitute a mechanism for elevating endogenous cytokinin levels. Although the N-glucosides (7- and 9- particularly) are generally inactive forms of cytokinins [126], [9G]BA has been found similarly active to free BA in retarding the senescence of radish leaf discs [122].

In summary, the generally low activity of [9G]BA in a variety of cytokinin bioassays indicates that this compound is probably an inactivation or detoxification form.

8.4 *The glucose-ribose conjugate – [9R-G]BA*

A new hexose (probably glucose) conjugate of [9R]Z, susceptible to β-glucosidase cleavage, was detected in Douglas-fir (*Pseudotsuga menziesii*) [152]. Immunoaffinity and mass spectral techniques indicated that this compound is not (OG)[9R]Z. It was suggested that the hexose moiety is attached to the purine ring or to the ribose group. The latter position was favoured owing to the ease of hydrolysis by β-glucosidase. However, N-conjugation does not totally preclude hydrolysis by β-glucosidase [221]. Earlier, Taylor et al. [202] had detected a novel ribosyl zeatin glycoside which could be the same compound further characterised by Morris et al. [152], but in a different coniferous species (*Pinus radiata*). These workers suggested that the hexose moiety was glucose. Van Staden and Mallett [223] and later Van Staden and Bayley [215] detected a glucosylated form of [9G]BA following BA application to tomato shoots. Further structural characterisation of this unknown metabolite was not attempted.

A disaccharide of BA, 6-(benzylamino)-9-(glucosylribosyl)purine was identified by Blakesley et al. [21] as the major metabolite in *Gerbera jamesonii* callus. The exact position of the ribose-glucose linkage was not determined. Unaware of the report on *Gerbera*, Auer and Cohen [9] reported on [9R-G]BA formation in *Petunia* leaves, and proposed a linkage of the ribose at the 3-position to glucose at the terminal (1) position. Morris et al. [152] suggested a possible storage role for this metabolite. In citing the observed activity of [9R-G]Z in the soybean hypocotyl bioassay [201]. Auer and Cohen [9] suggested that [9R-G]BA may contribute to the pool of inter-convertible active cytokinins. As further circumstantial evidence, these authors noted that [9R-G]BA formation was associated with increased shoot organogenesis in *Petunia* explants.

The production of glucosylated ribosides of cytokinins by plants of such diverse taxonomic relation as the Pinaceae, Compositae and Solanaceae, indicates that these compounds may be commonly and widely produced by plants, albeit as minor metabolites.

8.5 *The benzyladenine – alanine conjugates [9Ala]BA*

Lupinic acid ([9Ala]Z), the alanine conjugate of zeatin, was first identified in *Lupinus angustifolia* seedlings [129], and later shown [173] to exhibit only very weak activity in the radish-cotyledon cytokinin bioassay. Lupinic acid was at that time 'the only plant product known in which an amino acid moiety is conjugated to a nitrogen atom of the purine ring'.

A fraction 'C', isolated by Dyson et al. [54] from soybean callus, was shown to be a stable, long-lived BA derivative with cytokinin activity. This compound was converted from 20–25% of the applied BA and was metabolically related to [9R-MP]BA. This metabolite was considered by Deleuze et al. [46] to be 6-benzylamino-7-glucofuranosylpurine. Later researchers [56] have questioned this earlier assumption, believing the compound to be the alanine conjugate of BA.

Letham et al. [125] and Elliott and Thompson [56] reported on the existence of [9Ala]BA in *Phaseolus* seedlings and soybean callus respectively. This conjugate was found as the principal metabolite of BA in soybean [24]. These authors considered [9Ala]BA to be an inactivated form of BA. As [9Ala]BA showed only slight senescence retarding activity, Letham et al. [122] came to a similar conclusion. The use of inhibitors of alanine conjugation [238] has provided further circumstantial evidence to support this notion. Zhang et al. [241] noted that derivatives of BA with a slowly cleaved substituent at N-9 (to yield free BA), could be more effective than BA in exerting cytokinin activity. Such a case may be when only one or a few cytokinin applications are practicable for evoking a response. In contrast to these observed activities, [9Ala]BA at high concentrations was almost as active as BA in the *Amaranthus* bioassay [122].

As BA is probably not a substrate for cytokinin oxidase in all plant systems alternative inactivation through N-conjugation may account for the accumulation of [9Ala]BA in soybean callus [122]. Zhang et al. [241] investigated the suppression of [9Ala]BA formation in order to enhance the senescence retarding activity of BA. Inhibition of [9Ala]BA formation was accompanied by a greater degree of N^6-benzyl cleavage, associated with the production of adenine and adenosine. Consequently, these authors suggested that N^6-sidechain cleavage and alanine conjugation are alternative mechanisms for BA inactivation in soybean leaves. The low cytokinin activity of the alanine conjugates is probably due to an inability of tissues to readily cleave the alanine moiety and release free cytokinin [122]. The alanine conjugate of BA was largely unmetabolised in soybean leaf discs; no BA formation was detected [239]. However, zeatin was shown to be released from [9Ala]Z, which would explain the observed minor activity of [9Ala]Z in the soybean bioassay [168]. This raises the possibility that alanine conjugates are a further storage form of cytokinin. Palni et al. [168] viewed the observed stability of the alanine conjugate of zeatin to result from its compartmentation, and therefore protection against the enzymes involved in sidechain cleavage.

In soybean, rapid metabolism of BA to [9Ala]BA was confined to the first 24 hours after application, and was associated with uptake of the supplied BA. Letham et al. [122] hypothesised that remaining BA may be sub-compartmented in the cell, separated from the action of inactivating enzymes. Such is the extent of alanine conjugation with BA in soybean leaves that BA analogues have been found more effective than BA in regarding soybean leaf senescence [240].

The question was posed by Letham et al. [125] as to whether alanine conjugates of cytokinins are confined to the Leguminosae. This BA conjugate has been identified from *Phaseolus vulgaris* [125], *Glycine max* [56], *Lupinus angustifolius* [129], and probably *Lupinus luteus* [71].

9. Enzymes specific for N-conjugation of cytokinins

With the exception of cytokinin oxidase [234] research into enzymes of cytokinin metabolism had not been reported until 1979. Entsch et al. [62] then reported on the preparation of one of two isozymes from radish cotyledons which glycosylated cytokinins.

Letham and Palni [121] cited three characterised and purified enzymes known to show specificity for cytokinins, namely *cytokinin oxidase*, *cytokinin-7-glucosyl transferase*, and *β-(9-cytokinin)-alanine synthase*. Since this review was published, several other cytokinin-specific enzymes have been identified. These include *zeatin reductase* [131] and a *cis-trans*-isomerase of zeatin [13]. The existence of a 'cytokinin-β-glycosidase' has also been reported, as has a *cytokinin O-xylosyltransferase* [205].

The metabolism of exogenously applied cytokinins, including BA [21, 220] indicates the presence of other, as yet uncharacterised enzyme systems.

9.1 *Cytokinin-7-glucosyl transferase*

Radish cotyledon extracts yielded a single enzyme system collectively known as cytokinin-7-glucosyl transferase comprised of two enzymes/isozymes. These converted BA into 7- and 9-glucosides when uridine diphosphate glucose (UDPG) was supplied as a glucose donor [58, 60]. Cytokinin-7-glucosyl transferase produced the two glucosides in different proportions; the major isozyme favoured production of the 7-glucoside, and the minor glucosyl transferase formed the 7- and 9-cytokinin glucosides in similar proportions [62]. Entsch and Letham [58] expressed surprise to find that the 7- and 9-glucosides were not formed by separate enzymes, especially considering the small size of the enzyme (46,000 daltons).

However, in view of the many systems in which cytokinin-9-glucosides are produced as the major metabolite [19, 97, 145], it is possible that a separate cytokinin-9-glucosyltransferase exists, or a 'cytokinin-7-glycosyltransferase' which forms both the 7- and 9-glucosides, yet favours production of the latter. As with cytokinin oxidase-type systems [31, 100], different enzymes or isozymes of cytokinin-7-glucosyltransferase with a similar function are likely to occur in a range of plant tissues.

Although a trace enzyme, the glucosyl transferase studied by Entsch et al. [62] could exert a regulatory role in metabolism since cytokinins occur in trace amounts, evoking key responses at the sub-nanomolar level.

Entsch et al. [62] considered that inhibitors of cytokinin-7-glucosyl transferase merit study, as 'a stable, effective, and specific inhibitor *in vitro* could be a valuable physiological tool and a means of elevating endogenous free cytokinin levels by suppressing formation of the very weakly active 7-glucosides. Several studies have been undertaken in this regard [55, 89, 169, 200]. Greater effort in this area could result in the development of regulatory mechanisms optimizing tissue culture systems.

9.2 *β-Glucosidase*

Hydrolysis of O-glucosides of zeatin-like cytokinins may function in controlling cytokinin activity. In this regard β-glucosidases would play an important role [212]. Until recently, non-specific β-glucosidases were considered to be involved in cytokinin metabolism. However, the existence of a specific 'cytokinin-β-glucosidase' has recently been reported. Estruch et al. [65] transformed tobacco tissues with a *rol* C oncogene from the T-DNA of *Agrobacterium rhizogenes*. This gene coded for a 'cytokinin-β-glucosidase' which was capable of hydrolysing [9G]BA to its free base. Despite this report, Kaminek [99] considered such hydrolases to be either absent or inactive in normal plant cells.

Almond β-glucosidase did not hydrolyse cytokinin 7- or 9-glucosides *in vitro* [50], although limited cleavage of [7G]Z has been reported in radish tissues [126]. The resistance to hydrolysis was presumed due to their C-N glycosidic linkages [91] although enzymic degradation of a similar bond in [3G]BA [220] remains unaccountable.

β-Glucosidases have functions unrelated to growth [95], so their activity in regard to cytokinin metabolism is not surprising. Hughes [95] found two distinct β-glucosidases produced by clover callus. It was shown that β-glucosidase activity and concentration varied both between plants and between organs of the same plant. Genetic variation and the environment were cited as causal factors of this. McCreight et al. [135] later found different forms of β-glucosidase in different plantorgans of the same species.

9.3 *β-(9-Cytokinin)-alanine synthase*

The enzyme which converts cytokinin base to considerably less active alanine conjugates is known as lupinic acid synthase or β-(9-cytokinin)-alanine synthase [241]. This enzyme is classed as C-N-ligase lupinic acid ([9Ala]Z) [59, 154]. Enzyme-catalysed formation of lupinic acid was determined by ^{14}C incorporation from O-acetyl-srine-3-^{14}C as a substrate into lupinic acid. Murakoshi et al. [154] demonstrated that enzymes from different plant species which catalysed the synthesis of β-substituted alanines from O-acetyl-L-serine had different specificities; not all enzymes recognised zeatin as a substrate.

β-(9-Cytokinin)-alanine synthase was isolated from developing lupin seeds by Entsch et al. [61]. In this report, a number of adenine derivatives were shown to serve as substrates, although preference was shown for compounds with high cytokinin activity, including BA and Kinetin. In the reverse direction, a small amount of base was formed [168]. Although indole auxins have a similar type of ring structure to purines, IAA was not a substrate for lupinic acid synthase [61].

10. Oxidative catabolism of benzyladenine

10.1 *Cytokinin sidechain cleavage*

Cytokinin activity is conferred on (intact) purine molecules through the possession of a suitably-structured N^6-sidechain [132, 226]. When this sidechain is (oxidatively) removed, relatively inactive degradation products are formed. Further loss of activity occurs on disruption of the adenine moiety [18, 191]. The cytokinin-specific enzyme responsible for such sidechain cleavage is cytokinin oxidase, an enzyme common to both lower [4] and higher [234] plants.

The activity associated with oxidative catabolism was detected in many early cytokinin metabolic studies, involving the degradation of cytokinin bases and ribosides of both naturally-occurring [37, 144, 161] and synthetic cytokinins [57, 68, 69, 72, 134]. Fox et al. [72] even found a 'benzoic acid-like compound' to be a product of the cleavage of BA. A re-investigation revealed this 'benzoic acid-like compound' to be the aldehyde sidechain cleavage product [24].

Since the initial isolation and characterisation of cytokinin oxidase from *Zea mays* [234], there has been general agreement that the presence of a double bond in the isopentenyl sidechain of a cytokinin renders it susceptible to oxidation. A great number of studies have reported on such oxidation [79, 96, 165, 168, 170, 214].

The converse view that cytokinins with saturated aliphatic (DHZ) or ring (kinetin or BA) sidechains do not serve as substrates for cytokinin oxidase, has also been widely advocated [86, 121, 126, 137, 160, 167, 234]. Despite regular reports on degradation product formation, cytokinin oxidase-mediated catabolism of both BA and kinetin remains controversial. This scepticism is well substantiated, for to date, no cytokinin oxidase preparations isolated from plants have appreciably utilised BA [29, 100]. Despite the lack of activity observed *in vitro*, a large body of circumstantial evidence has accumulated which is indicative of *in vivo* BA degradation. Many [8-^{14}C]-labelled products of oxidative catabolism (adenosine, adenine, adenine nucleotides, ureides) have been identified following application of [8-^{14}C]BA to a wide variety of plant systems [17, 54, 67, 72, 104, 134, 203, 216].

The degradation of BA and kinetin in many systems has been attributed to a separate enzyme system [121], distinct from cytokinin oxidase. These authors attributed the cleavage of furfuryl groups from the N^6-position (to yield adenine and its derivatives), to such a system. An alternative theory for observed loss of the benzyl group was proposed by McCalla et al. [134]. They considered that C-8 from [8-^{14}C]BA could be lost to the 'one carbon' pool with subsequent reincorporation into newly synthesised purine. Some 12 years prior to Whitty and Hall's report of cytokinin oxidase [234], McCalla et al. [134] also considered the possibility of direct enzymic removal of the benzyl group.

Zhang and Letham [239] hypothesised that N^6 de-benzylation of BA 'probably involves an imino intermediate formed enzymically by elimination of a hydrogen atom from both the NH group at position 6 and the benzylic methylene'. In species where cytokinin oxidase catabolise BA [67, 239], it is possible that such activity can only be expressed if the enzyme facilitating production of the imino form is also present and active. The imino-purine intermediate postulated by Whitty and Hall [234] has been isolated [31] as an intermediate in the degradation of isopentenylated cytokinins. As BA has not been shown to be a substrate for cytokinin oxidases in any *in vitro* assays to date [100], the importance of an enzyme catalysing formation of an imino intermediate should be accorded more consideration.

Chatfield and Armstrong [31] provided an indication that distinct isozymes of cytokinin oxidase may exist. Cytokinin oxidase from *Vinca rosea* crown gall tissues [137] appeared to be a different system to that partially purified from maize kernels [234]. The molecular weights of the two enzymes, determined by gel filtration, are very different: 944400 (± 10%) for maize and 25100 (± 10%) for *Vinca rosea*. The two enzymes exhibited similar substrate specificities; neither recognised BA as a substrate. Thus, evidence for heterogeneity in cytokinin oxidase activity is provided by the range of molecular weight estimates for the enzyme from different plant tissues. Chatfield and Armstrong [31] suggested that this heterogeneity is related to glycosylation and noted that should this be confirmed, then new implications for the compartmentation and regulation of this enzyme would arise. A clear implication of such heterogeneity is that specific cytokinin oxidases may exist which preferentially attack BA, rather than isopentenylated cytokinins. In all studies to date, *in vitro* assays have been performed with enzymes extracted from tissues in which low *in vivo* degradation has been observed. Enzyme extracts from tissues which are known to substantially degrade exogenously

applied BA e.g. *Glycine max* cv. Acme) [67] could well prove rewarding in this regard.

Cytokinin oxidase is able to utilise a number of different cytokinin substrates, including bases, ribosides [190], N-glycosides and N-alanyl conjugates [137]. However, ribonucleotide [110, 137] and O-glucoside [186, 225] forms are thought to be resistant to cytokinin oxidase. Data has been presented which suggests that substrate induction occurs, where cytokinin degradation is promoted by cytokinins themselves [31, 153, 203]. The exact metabolic level of sidechain removal from BA is not known [105]. Several studies have shown that N-conjugates are susceptible to further inactivation through oxidation. Cytokinin oxidase is considered to be the active catalyst. Soybean callus degraded [7G]Z, [9G]Z, and [9Ala]Z to [7G]Ade, [9G]Ade and [9Ala]Ade respectively [168]. These compounds appeared as minor metabolites. Prior to this report, Letham et al. [126] had identified [7G]Ade in radish seedlings. The 7-glucoside of zeatin was metabolised to adenine, adenosine and [7G]Ade within two days [138]. Should a cytokinin oxidase-type system fully utilise BA metabolites, one might expect these same products to be produced.

A further metabolite of BA may be adenosine-5^{1}-phosphate (AMP), which has previously been identified during metabolic studies with zeatin [52] and [7G]Z [138]. Early BA metabolic studies [47, 134] reported on the identification of adenylic nucleotides as products. However, in recent years, no reports of AMP formation from BA have been published.

McGaw and Horgan [137] considered cytokinin oxidase as a candidate for the control of endogenous cytokinin species and levels. However, Whitty and Hall [234] had earlier cautioned against assuming that the whole purpose of cytokinin oxidase is to help maintain some specific level of cytokinins. Rather, these authors viewed the rate of turnover of cytokinins to be a means of conveying information necessary for control of cellular growth. An actual role for cytokinin oxidase in the control of the endogenous levels of cytokinins is difficult to assign, when one considers certain anomalies. In *Vinca* crown gall tissue and corn kernels, the most abundant cytokinins are [9R]Z and zeatin [185]. Both these hormones are readily metabolised by cytokinin oxidase [234], the presence of which has been demonstrated in these tissues. Compartmentation of substrates probably prevents these cytokinins coming in contact with the oxidative enzyme system [137]. Evidence for such distinct compartmentation of different isozymes of cytokinin oxidase has recently been presented [100].

Plant cytochrome P-450 found in the microsomal fraction of cauliflowers was shown by Chen and Leisner [36] to exhibit oxidative dealkylation activity, much as cytokinin oxidase does. After two hours incubation of [9R]iP with cytochrome P-450, about 15–25% adenosine was formed.

If therefore appears that benzyladenine is the substrate for a cytokinin-oxidase type system which degrades this cytokinin adenine. Cytokinin oxidase may not directly utilise BA, but only following conversion to an imino intermediate. The potentially restricted distribution of an (uncharacterised) enzyme catalysing formation of this intermediate, would account for the limited degradation of BA (relative to zeatin or iP) which has been observed in many tissues.

11. Conclusion

Cytokinin metabolites possess functional, though somewhat obscure roles in plants [121, 229], contributing to either an active or inactive pool. Inactivation of cytokinin occurs through sidechain cleavage or alternatively N-conjugation, which proceeds through 9-alanylation or 7- and/or 9-glucosylation [99, 138]. The 3-glucoside is more biologically active than other cytokinin N-glucosides [122, 127] and appears reversibly sequestered [119, 220], suggesting a storage role. Internal levels of free, non-metabolised base appear important in the initiation of physiological responses [207]. Nucleosides and nucleotides are also considered as active forms [109, 121], given their ready conversion to cytokinin bases and/or interconversion [215, 221].

From a physiological viewpoint, cytokinin metabolism may be classified [93] under three headings:

1. Irreversible loss of biological activity through oxidative degradation of the N^6 sidechain (products here referred to as 'oxidation products').
2. Irreversible conjugation with alanine or glucose with loss of, or reduction in activity (products here referred to as 'N-conjugates').
3. Reversible conjugation to (inter-convertible) compounds which are themselves active, or serve as storage forms which may be converted to active cytokinins (her referred to as the 'active pool').

References

1. Adedipipe NO, Hunt LA and Fletcher RA (1971) Effects of benzyladenine on photosynthesis, growth and senescence of the bean plant. Physiol Plant 25: 151–153
2. Agrawal KML and Bahl OP (1968) Glycosidases of *Phaseolus vulgaris*. II. Isolation and general properties. J Biol Chem 243: 103–111
3. Ainley WM, McNeil KJ, Hill JW, Lingle WL, Simpson RB, Brenner ML, NaGao RT and Key JL (1993) Regulatable endogenous production of cytokinins up to 'toxic' levels in transgenic plants and plant tissues. Plant Mol Biol 22: 13–23
4. Armstrong DJ and Firtel RA (1989) Cytokinin oxidase activity in the cellular slime mold, *Dictyostelium discoideum*. Dev Biol 136: 491–499
5. Armstrong DJ, Murai N, Taller BJ and Skoog F (1976) Incorporation of a cytokinin N^6-benzyladenine into tobacco callus transfer ribonucleic acid and ribosomal nucleic acid preparations. Plant Physiol 57: 15–22
6. Ashford AE (1970) Histochemical localisation of β-glycosidases in roots of *Zea mays*. I. A simultaneous coupling azo-dye technique for the localisation of β-glycosidase and β-galactosidase. protoplasma 71: 281–293
7. Ashford AE and McCully ME (1970) Histochemical localisation of β-glycosidases in roots of *Zea mays*. II. Changes in localisation and activity of β-glucosidase in main root apex. protoplasma 71: 389–402
8. Ashihara H (1983) Changes in activities of purine salvage and ureide synthesis during germination of black gram (*Phaseolus mungo*) seeds. Z Pflanzenphysiol 113: 47–60
9. Auer CA and Cohen JD (1993) Identification of a benzyladenine disaccharide conjugate produced during shoot organogenesis in *Petunia* leaf explants. Plant Physiol 102: 541–545
10. Auer CA, Cohen JD, Laloue M and Cooke TJ (1992) Comparison of benzyl adenine metabolism in two *Petunia hybrida* lines differing in shoot organogenesis. Plant Physiol 98: 1035–1041
11. Auer CA, Laloue M, Cohen JD and Cooke TJ (1992) Benzyladenine uptake and metabolism during *Petunia* shoot organogenesis. In: Kaminek M, Mok DWS and Zazimalova E (eds) Physiology and Biochemistry of Cytokinins in Plants, pp 69–70. The Hague, The Netherlands: SPB Academic Publishing
12. Auer CA, Laloue M, Cohen JD and Cooke TJ (1992) Uptake and metabolism of benzyladenine during shoot organogenesis in *Petunia* leaf explants. Plant Growth Regul 11: 105–114
13. Bassil NV, Mok DWS and Mok MC (1993) Partial purification of a *cis-trans*-isomerase of zeatin from immature seed of *Phaseolus vulgaris* L. Plant Physiol 102: 867–872
14. Bayley AD, Van Staden J, Mallett JA and Drewes SE (1989) The *in vitro* metabolism of [8-^{14}C]benzyladenine by excised organs of tomato plants. Plant Growth Regul 8: 193–204
15. Becker JL and Roussaux J (1981) 6-Benzyladenine as a growth factor for *Drosophila melanogaster* cells grown in vitro. In: Guern J and Peaud-Lenoël C (eds) Metabolism and Molecular Activities of Cytokinins, pp 319–329. Berlin, Germany: Springer-Verlag
16. Bezemer-Sybrandy SM and Veldstra H (1971) The metabolism of 6-benzylaminopurine in *Lemna minor*. Physiol Plant 25: 1–7
17. Biondi S, Canciani L and Bagnni N (1984) Uptake and translocation of benzyladenine by elm shoots cultured *in vitro*. Can J Bot 62: 2385–2390
18. Black MK and Osborne DJ (1965) Polarity of transport of benzyladenine, adenine and indole-3-acetic acid in petiole segments of *Phaseolus vulgaris*. Plant Physiol 40: 676–680
19. Blakesley D (1991) Uptake and metabolism of 6-benzyladenine in shoot cultures of *Musa* and *Rhododendron*. Plant Cell Tissue Org Cult 25: 69–74
20. Blakesley D and Constantine D (1992) Uptake and metabolism of 6-benzyladenine in shoot cultures of a range of species. Plant Cell Tissue Org Cult 28: 183–186
21. Blakesley D, Lenton JR and Horgan R (1990) Benzyladenine ribosylglucoside: a metabolite of benzyladenine in *Gerbera jamesonii*. Phytochem 30: 387–388
22. Bopp M and Ercichsen U (1981) Metabolism of the cytokinins in mosses. In: Guern J and Peaud-Lenoël C (eds) Metabolism and Molecular Activities of Cytokinins, pp 105–114. Berlin, Germany: Springer-Verlag
23. Brnnegar AC, Cooper G, Stevens A, Hauer CR, Shabanowitz J, Hunt DF and Fox JE (1988) Characterisation of a benzyladenine binding-site peptide isolated from a wheat cytokinin-binding protein: sequence analysis and identification of a single affinity-labeled histidine residue by mass spectroscopy. Proc Natl Acad Sci USA 85: 5927–5931
24. Brownlee BG, Hall RH and Whitty CD (1975) 3-Methyl-2-butenal: an enzymic product of the cytokinin, N^6-(Δ^2-isopentenyl)adenine. Can J Biochem 53: 37–41
25. Bui-Dang-Ha D and Nitsch JP (1970) Isolation of zeatin riboside from the chicory root. Planta 95: 119–126
26. Burch LR and Stuchbury T (1986) Purification and properties of adenosine nucleosidase from tomato (*Lycopersicon esculentum*) roots and leaves. J Plant Physiol 125: 267–273
27. Burch LR and Stuchbury T (1987) Activity and distribution of enzymes that interconvert purine bases, ribosides and ribotides in the tomato plant and possible implications for cytokinin metabolism. Physiol Plant 69: 283–288
28. Caers M, Rudelsheim P, Van Onckelen H and Horemans S (1985) Effect of heat stress on photosynthetic activity and chloroplast ultrastructure in correlation with endogenous cytokinin concentration in maize seedlings. Plant Cell Physiol 26: 47–52
29. Chatfield JM and Armstrong DJ (1986) Regulation of cytokinin oxidase in callus tissues of *Phaseolus vulgaris* L. cv. Great Northern. Plant Physiol 80: 493–499
30. Chatfield JM and Armstrong DJ (1987) Cytokinin oxidase from *Phaseolus vulgaris* callus tissues. Enhanced *in vitro* activity of the enzyme in the presence of copper-imidazole complexes. Plant Physiol 84: 726–731
31. Chatfield JM and Armstrong DJ (1988) Cytokinin oxidase from *Phaseolus vulgaris* callus tissues. Plant Physiol 88: 245–247
32. Chen C-M (1981) Biosynthesis and enzymic regulation of the interconversion of cytokinin. In: Guern J and Peaud-Lenoël C (eds) Metabolism and Molecular Activities of Cytokinins, pp 34–43. Berlin, Germany: Springer-Verlag
33. Chen C-M and Eckert RL (1977) Phosphorylation of cytokinin by adenosine kinase from wheat germ. Plant Physiol 59: 443–447
34. Chen C-M and Kristopeit SM (1981) Metabolism of cytokinin: deribosylation of cytokinin ribonucleoside by adenine nucleosidase from wheat germ cells. Plant Physiol 68: 1020–1023
35. Chen C-M and Kristopeit SM (1981) Metabolism of cytokinin: dephosphorylation of cytokinin ribonucleotide by 5′-nucleotidases from wheat germ cytosol. Plant Physiol 67: 494–498

17 9 23

36. Chen C-M and Leisner SM (1984) Modification of cytokinins by cauliflower microsomal enzymes. Plant Physiol 75: 442–446
37. Chen C-M, Logan DM, McLennan BD and Hall RH (1968) Studies on the metabolism of a cytokinin, N^6-(Δ^2-isopentenyl)-adenosine. Plant Physiol 43(Suppl.): 18
38. Chen C-M, Melitz DK and Clough FW (1982) Metabolism of cytokinin: phosphoribosylation of cytokinin bases by adenine phosphoribosyltransferase from wheat germ. Arch Biochem Biophys 214: 634–641
39. Chen C-M and Petschow B (1978) Metabolism of cytokinin: ribosylation of cytokinin base by adenosine phosphorylase from wheat germ. Plant Physiol 62: 871–874
40. Chism GW, Long AR and Rolle R (1984) Assay of enzymes involved in cytokinin metabolism by means of reversed-phase high performance liquid chromatography. J Chrom 317: 263–270
41. Cowley DE, Duke CC, Liepa AJ, Macleod JK and Letham DS (1978) The structure and synthesis of cytokinin metabolites. I. The 7- and 9-β-D-glucofuranosides and pyranosides of zeatin and 6-benzylaminopurine. Aust J Chem 31: 1095–1111
42. Datko AH and Maclachlan GA (1970) Patterns of development of glycosidase activities in the pea epicotyl. Can J Bot 48: 1165–1169
43. Davey JE and Van Staden J (1976) Cytokinin translocation: changes in zeatin and zeatin riboside levels in the root exudate of tomato plants during their development. Planta 130: 69–72
44. Dei M (1983) Benzyladenine-induced stimulation of chlorophyll formation in attached cotyledons of etiolated cucumber seedlings. Plant Sci Lett 30: 251–257
45. Dekhuijzen HM, Bossen ME and Vonk CR (1978) The uptake of cytokinins by protoplasts and root sections of *Brassica campestris*. Physiol Plant 43: 142–147
46. Deleuze GG, McChesney JD and Fox JE (1972) Identification of a stable cytokinin metabolite. Biochem Biophys Res Commun 48: 1426–1432
47. Doree M and Guern J (1973) Short-time metabolism of some exogenous cytokinins in *Acer pseudoplatanus* cells. Biochim Biophys Acta 304: 611–622
48. Doree M and Terrine C (1973) Enzymatic synthesis of ribonucleoside-5'-phosphate from some N^6-substituted adenosines. Phytochem 12: 1017–1023
49. Doree M, Terrine C and Guern J (1972) Plant cell permeability to kinetin. In: Hormonal Regulation in Plant Growth Development, pp 221–231. Weinheim, Germany: Verlag Chemie
50. Duke CC, Letham DS, Parker CW, Macleod JK and Summons RE (1979) The complex of O-glucosylzeatin derivatives formed in *Populus* species. Phytochem 18: 819–824
51. Duke CC, Liepa AJ, Macleod JK, Letham DS and Parker CW (1975) Synthesis of raphanatin and its 6-benzylaminopurine analogue. J Chem Soc Chem Commun 1146
52. Duke CC, Macleod JK, Summons RE, Letham DS and Parker CW (1978) The structure and synthesis of cytokinin metabolites. II. Lupinic acid and O-β-D-glucopyranosyl zeatin from *Lupinus angustifolius*. Aust J Chem 31: 1291–1301
53. Dybing CD and Lay C (1981) Field evaluations of morphactins and other growth regulators for senescence delay of flax, soybean, wheat and oats. Crop Sci 21: 879–884
54. Dyson W, Fox JE and McChesney JD (1972) Short term metabolism of urea and purine cytokinins. Plant Physiol 49: 506–513
55. Elliott DC and Murray AW (1975) Evidence against an involvement of cyclic nucleotides in the induction of betacyanin synthesis by cytokinins. Biochem J 146: 333–337
56. Elliott DC and Thompson MJ (1982) The identity of the major metabolite of benzylaminopurine in soybean cultures and the inhibition of its formation by aminophylline. Plant Sci Lett 28: 29–38
57. El-Saidi MT (1971) Transport and metabolism of kinetin-8-^{14}C in *Zea mays* L. roots. Ann Bot 35: 1073–1083
58. Entsch B and Letham DS (1979) Enzymic glucosylation of the cytokinin, 6-benzylaminopurine. Plant Sci Lett 14: 205–212
59. Entsch B, Letham DS, Parker CW, Summons RE and Gollnow BI (1979) Metabolites of cytokinins. In: Skoog F (ed) Plant Growth Substances 1979, pp 109–118. Berlin, Germany: Springer Verlag
60. Entsch B, Parker CW and Letham DS (1978) Enzymatic glucosylation of the phytohormone, zeatin. Aust Biochem Soc Proc 11: 53
61. Entsch B, Parker CW and Letham DS (1983) An enzyme from lupin seeds forming alanine derivatives of cytokinins. Phytochem 22: 375–381
62. Entsch B, Parker CW, Letham DS and Summons RE (1979) Preparation and characterization using high performance liquid chromatography, of an enzyme forming glucosides of cytokinins. Biochim Biophys Acta 570: 124–139
63. Erichsen U, Knoop B and Bopp M (1978) Uptake, transport and metabolism of cytokinin in moss protonema. Plant Cell Physiol 19: 839–850
64. Ernst D, Schäfer W and Oesterholt D (1983) Isolation and identification of a new, naturally occurring cytokinin (6-benzyl-aminopyrine riboside) from an anise cell culture (*Pimpinella anisum* L.). Planta 159: 222–225
65. Estruch JJ, Chriqui D, Grossmann K, Schell J and Spena A (1991) The plant oncogene *rolC* is responsible for the release of cytokinins from glucoside conjugates. EMBO J 10: 2889–2895
66. Fletcher RA (1969) Retardation of leaf senescence by benzyladenine in intact bean plants. Planta 89: 1–8
67. Forsyth C and Van Staden J (1986) The metabolism and cell division activity of adenine derivatives in soybean callus. J Plant Physiol 124: 275–287
68. Fox JE (1964) Incorporation of kinins into the RNA of plant tissue cultures. Plant Physiol 39(Suppl.): 31
69. Fox JE (1966) Incorporation of a kinin, *N*,6-benzyladenine, into soluble RNA. Plant Physiol 41: 75–82
70. Fox JE and Chen C-M (1967) Characterisation of labeled ribonucleic acid from tissue grown on ^{14}C-containing cytokinins. J Biol Chem 242: 4490–4494
71. Fox JE, Comette J, Deleuze G, Dyson W, Giersak C, Niu P, Zapata J and McChesney J (1973) The formation, isolation, and biological activity of a cytokinin 7-glucoside. Plant Physiol 52: 627–632
72. Fox JE, Dyson W, Sood C and McChesney JD (1972) Active forms of the cytokinins. In: Carr DJ (ed) Plant Growth Substances 1970, pp 449–458. Berlin, Germany: Springer-Verlag
73. Fox JE, Sood CK, Buckwalter B and McChesney JD (1971) The metabolism and biological activity of a 9-substituted cytokinin. Plant Physiol 47: 275–281
74. Fox JE, Sood CK and McChesney JD (1973) Effect of substituents at the 9-position on cytokinin activity. Phytochem 12: 1531–1533
75. Fusseder A, Ziegler P, Peters W and Beck E (1989) Turnover of O-glucosides of dihydrozeatin and dihydrozeatin-9-riboside during the cell growth cycle of photoautotrophic cell suspension cultures of *Chenopodium rubrum*. Bot Acta 102: 335–340

76. Garrison FR, Brinker AM and Noodén LD (1984) Relative activities of xylem-supplied cytokinins in retarding soybean leaf senescence and sustaining pod development. Plant Cell Physiol 25: 213–224
77. Gawer M, Laloue M, Terrine C and Guern J (1977) Metabolism and biological significance of benzyladenine-7-glucoside. Plant Sci Lett 8: 267–274
78. Gilbert ML, Thompson JE and Dumbroff BE (1980) Delayed cotyledon senescence following treatment with a cytokinin: an effect at the level of membranes. Can J Bot 58: 1797–1803
79. Gordon ME, Letham DS and Parker CW (1974) The metabolism and translocation of zeatin in intact radish seedlings. Ann Bot 38: 809–825
80. Guern J, Doree M and Sadorge P (1968) Transport, metabolism and biological activity of some cytokinins. In: Wightman F and Setterfield G (eds) Biochemistry and Physiology of Plant Growth Substances, pp 1155–1167. Ottawa, Canada
81. Guranowski A and Schneider Z (1977) Purification and characterisation of adenosine nucleosidase from barley leaves. Biochim Biophys Acta 482: 145–158
82. Hamill JD (1993) Alterations in auxin and cytokinin metabolism of higher plants due to expression of specific genes from pathogenic bacteria: A review. Aust J Plant Physiol 20: 405–423
83. Hecht SM, Bock RM, Schmitz RY, Skoog F, Leonard NJ and Occolowitz J (1971) Question of the ribosyl moiety in the promotion of callus growth by exogenously added cytokinins. Biochem 10: 4224–4228
84. Hecht SM, Frye RB, Werner D, Hawrelak SD, Skoog F and Schmitz RY (1975) On the 'activation' of cytokinins. J Biol Chem 250: 7343–7351
85. Hendry NS, Van Staden J and Allan P (1982) Cytokinins in *Citrus*. II. Fluctuations during growth in juvenile and adult plants. Sci Hort 17: 247–256
86. Henson IE (1978) Types, formation and metabolism of cytokinins in leaves of *Alnus glutinosa* (L.) Gaertn. J Exp Bot 29: 935–951
87. Henson IE and Wareing PF (1977) Cytokinins in *Xanthium strumarium* L: the metabolism of cytokinins in detached leaves and buds in relation to photoperiod. New Phytol 78: 27–33
88. Henson IE and Wheeler CT (1977) Hormones in plants bearing nitrogen-fixing root nodules: metabolism of [8-^{14}C]zeatin in root nodules of *Alnus Glutinosa* (L.) Gaertn. J Exp Bot 28: 1087–1098
89. Hocart CH, Letham DS and Parker CW (1991) Substituted xanthines and cytokinin analogues as inhibitors of cytokinin *N*-glucosylation. Phytochem 30: 2477–2486
90. Hochstadt-Ozer J and Stadtman ER (1971) Adenine phosphoribosyl-transferase in isolated membrane preparations and its role in transport of adenine across the membrane. J Biol Chem 246: 5304–5311
91. Horgan R (1975) A new cytokinin metabolite. Biochem Biophys Res Commun 65: 358–363
92. Horgan R (1984) Cytokinins. In: Wilkins MB (ed) Advanced Plant Physiology, pp 53–75. Bath, UK: The Pitman Press
93. Horgan R (1987) Cytokinin genes. In: Hoad GV, Lenton JR, Jackson MB and Atkin RK (eds) Hormone Action in Plant Development, pp 119–130. London, UK: Butterworths
94. Horgan R, Hewett EW, Purse JG and Wareing PF (1973) A new cytokinin from *Populus robusta*. Teth Lett 30: 2827–2828
95. Hughes MA (1968) Studies on β-glucosidase production in cultured tissue of *Trifolium repens* L. J Exp Bot 19: 52–63
96. Hutton MJ and Van Staden J (1984) Transport and metabolism of labelled zeatin applied to the stems of *Phaseolus vulgaris* at different stages of development. Z Pflanzenphysiol 114: 331–339
97. Jones LH and Hanke DE (1992) Kinetin-9-glucoside is a major metabolite of kinetin in oil palm cultures. In: Kaminek M, Mok DWS and Zazimalova E (eds) Physiology and Biochemistry of Cytokinins in Plants, pp 65–68. The Hague, The Netherlands: SPB Academic Publishing
98. Jouanneau J-P and Teyssendier de la Serve B (1981) Covalent insertion of N^6-benzyladenine into rRNA species of tobacco cells. In: Guern J and Peaud-Lenoël C (eds) Metabolism and Molecular Activities of Cytokinins, pp 228–238. Berlin, Germany: Springer-Verlag
99. Kaminek M (1992) Progress in cytokinin research. Tibtech 10: 159–164
100. Kaminek M and Armstrong DJ (1990) Genotypic variation in cytokinin oxidase from *Phaseolus* callus cultures. Plant Physiol 93: 1530–1538
101. Krenitsky TA, Papioannou R and Elion GB (1969) Human hypoxanthine phosphoribosyltransferase. II. Kinetics and chemical modification. J Biol Chem 244: 1271–1277
102. Kuiper D, Kuiper PJC, Lambers H, Schuit J and Staal M (1989) Cytokinin concentration in relation to mineral nutrition and benzyladenine treatment in *Plantago major* ssp. *pleiosperma*. Physiol Plant 75: 511–517
103. Laloue M (1977) Cytokinins: 7-glucosylation is not a prerequisite of the expression of their biological activity. Planta 134: 273–275
104. Laloue M and Pethe C (1982) Dynamics of cytokinin metabolism in tobacco cells. In: Wareing PF (ed) Plant Growth Substances 1982, pp 185–195. New York, USA: Academic Press
105. Laloue M, Pethe-Terrine C and Guern J (1981) Uptake and metabolism in tobacco cells: studies in relation to the expression of their biological activities. In: Guern J and Peaud-Lenoël C (eds) Metabolism and Molecular Activities of Cytokinins, pp 80–96. Berlin, Germany: Springer-Verlag
106. Laloue M, Terrine C and Gawer M (1974) Cytokinins: formation of the nucleoside-5′-triphosphate in tobacco and *Acer* cells. Fed Eur Biochem Soc Lett 46: 45–50
107. Laloue M, Terrine C and Guern J (1977) Cytokinins: metabolism and biological activity of N^6-(Δ^2-isopentenyl) adenosine and N^6-(Δ^2-isopentenyl)adenine in tobacco cells and callus. Plant Physiol 59: 478–483
108. Lampugnani MG, Fantelli R, Longo GP, Longo CP and Rossi G (1981) Uptake of benzyladenine by excised watermelon cotyledons. Plant Physiol 68: 11–14
109. Lee D and Moffatt BA (1993) Purification and characterisation of adenine phosphoribosyltransferase from *Arabidopsis thaliana*. Physiol Plant 87: 483–493
110. Lee YH, Mok MC, Mok DWS, Griffin DA and Shaw G (1985) Cytokinin metabolism in *Phaseolus* embryos: genetic difference and the occurrence of novel zeatin metabolites. Plant Physiol 77: 635–641
111. Le Floc' HF, Lafleuriel J and Guillot A (1978) L'adeninephospho-ribosyltransferase des pousses de topinambour *Helianthus tuberosus*. Phytochem 17: 643–646
112. Le Floc' HF, Lafleuriel J and Guillot A (1982) Interconversion of purine nucleotides in Jerusalem artichoke shoots. Plant Sci Lett 27: 309–316

19 8 23

113. Leonard NJ, Hecht SM, Skoog F and Schmitz RY (1968) Cytokinins: synthesis of 6-(3-methyl-3-butenylamino)-β-D-ribofuranosyl purine (3iPA) and the effect of sidechain unsaturation on the biological activity of isopentylaminopurines and their ribosides. Proc Natl Acad Sci USA 59: 15–21
114. Leonard NJ, Hecht SM, Skoog F and Schmitz RY (1969) Cytokinins: synthesis, mass spectra, and biological activity of compounds related to zeatin. Proc Natl Acad Sci USA 63: 175–182
115. Leopold AC and Kawase M (1964) Benzyladenine effects on leaf growth and senescence. Am J Bot 51: 294–298
116. Leszczynska D, Schneider Z, Tomaszewski M and Mackowiak M (1984) Comparative studies on adenosine nucleosidase occurrence in plants. Ann Bot 54: 847–849
117. Letham DS (1973) Regulators of cell division in plant tissues. XV. Cytokinins from *Zea mays*. Phytochem 12: 245–2455
118. Letham DS (1978) Cytokinins. In: Letham DS, Goodwin PB and Higgins TJV (eds) Phytohormones and Related Compounds – a Comprehensive Treatise. Volume 1, pp 205–263. Amsterdam, The Netherlands: Elsevier Press
119. Letham DS and Gollnow BI (1985) Regulators of cell division in plant tissues. XXX. Cytokinin metabolism in relation to radish cotyledon expansion and senescence. Plant Growth Regul 4: 129–145
120. Letham DS, Gollnow BI and Parker CW (1979) The reported occurrence of 7-glucofuranoside metabolites of cytokinins. Plant Sci Lett 15: 217–223
121. Letham DS and Palni LMS (1983) The biosynthesis and metabolism of cytokinins. Annu Rev Plant Physiol 34: 163–197
122. Letham DS, Plani LMS, Tao G-Q, Gollnow BI and Bates CM (1983) Regulators of cell division in plant tissues. XXIX. The activities of cytokinin glucosides and alanine conjugates in cytokinin bioassays. Plant Growth Regul 2: 103–115
123. Letham DS, Parker CW and Gordon ME (1972) Regulators of cell division in plant tissues. XIV. The cytokinin activities and metabolism of 6-acylaminopurines. Physiol Plant 27: 285–290
124. Letham DS, Summons RE, Entsch R, Gollnow BI, Parker CW and Macleod JK (1978) Regulators of cell division in plant tissues. XXVI. Glucosylation of cytokinin analogues. Phytochem 17: 2053–2057
125. Letham DS, Summons RE, Parker CW and Macleod JK (1979) Regulators of cell division in plant tissues. XXVII. Identification of an amino-acid conjugate of 6-benzylaminopurine formed in *Phaseolus vulgaris* seedlings. Planta 146: 71–74
126. Letham DS, Tao G-Q and Parker CW (1982) An overview of cytokinin metabolism. In: Wareing PF (ed) Plant Growth Substances 1982, pp 143–153. London, UK: Academic Press
127. Letham DS, Wilson MM, Parker CW, Jenkins ID, Macleod JK and Summons RE (1975) Regulators of cell division in plant tissues. XXIII. The identity of an unusual metabolite of 6-benzylaminopurine. Biochim Biophys Acta 399: 61–70
128. Lindoo SJ and Noodén LD (1978) Correlations of cytokinins and abscisic acid with monocarpic senescence in soybean. Plant Cell Physiol 19: 997–1006
129. Macleod JK, Summons RE, Parker CW and Letham DS (1975) Lupinic acid, a purinyl amino acid and a novel metabolite of zeatin. J Chem Soc Commun: 865
130. Martin JH (1969) A study of cytokinin metabolism. Ph.D. Thesis. Diss Abstr B, Sci Eng 30: 1000–1001
131. Martin RC, Mok MC, Shaw G and Mok DWS (1989) An enzyme mediating the conversion of zeatin to dihydrozeatin in *Phaseolus* embryos. Plant Physiol 90: 1630–1635
132. Matsubara S (1990) Structure-activity relationships of cytokinins. Crit Rev Plant Sci 9: 17–57
133. Matsubara S, Shiojiri S, Fujii T, Ogawa D, Inamura K, Yamagishi K and Koshimizu K (1977) Synthesis and cytokinin activity of (R)- and (S)- dihydrozeatins and their ribosides. Phytochem 16: 933–937
134. McCalla DR, Morré DJ and Osborne DJ (1962) The metabolism of a kinin, benzyladenine. Biochim Biophys Acta 55: 522–528
135. McCreight JD, Pharr DM, Lower RL and Sox HN (1976) Comparative study of β-glucosidase from cotyledons and fruits of cucumber, *Cucumis sativus*. Physiol Plant 37: 17–22
136. McGaw BA, Heald JK and Horgan R (1984) Dihydrozeatin metabolism in radish seedlings. Phytochem 23: 1373–1377
137. McGaw BA and Horgan R (1983) Cytokinin oxidase from *Zea mays* kernels and *Vinca rosea* crown gall tissue. planta 159: 30–37
138. McGaw RA and Horgan R (1985) Cytokinin metabolism and the control of cytokinin activity. Biol Plant 27: 180–187
139. Miernyk JK and Blaydes DF (1977) Short-term metabolism of radioactive kinetin during lettuce seed germination. Pysiol Plant 39: 4–8
140. Miller CO (1967) Cytokinins in *Zea mays*. Ann New York Acad Sci 144: 251–257
141. Miller CO (1968) Naturally-occurring cytokinins. In: Wightman F and Setterfield G (eds) Biochemistry and Physiology of Plant Growth Substances, pp 33–45. Ottawa, Canada: Runge Press
142. Miller GW and Evans HJ (1955) Nucleosidase from higher plants. Plant Physiol 30(Suppl.): 37
143. Minocha SC and Nissen P (1982) Uptake of benzyladenine by tuber slices of Jerusalem artichoke (*Helianthus tuberosus* L.) over a wide concentration range. Plant Physiol 70: 528–531
144. Miura G and Hall RH (1973) *Trans*-ribosylzeatin. Its biosynthesis in *Zea mays* endosperm and the mycorrhizal fungus *Rhizopogon roseolus*. Plant Physiol 51: 563–569
145. Moffatt B, Pethe C and Laloue M (1991) Metabolism of benzyladenine is impaired in a mutant of *Arabidopsis thaliana* lacking adenine phosphoribosyltransferase activity. Plant Physiol 95: 900–908
146. Mok MC, Martin RC, Mok DWS and Shaw G (1992) Cytokinin activity, metabolism and function in *Phaseolus*. In: Kaminek M, Mok DWS and Zazimalova E (eds) Physiology and Biochemistry of Cytokinins in Plants, pp 41–46. The Hague, The Netherlands: SPB Academic Publishing
147. Mok DWS and Mok MC (1987) Metabolism of ^{14}C-zeatin in *Phaseolus* embryos: occurrence of O-xylosyldihydrozeatin and its ribonucleoside. Plant Physiol 84: 596–599
148. Mok MC, Mok DWS, Dixon SC, Armstrong DJ and Shaw G (1982) Cytokinin structure-activity relationships and the metabolism of N^6-(Δ^2-isopentenyl)adenosine-[8-^{14}C] in *Phaseolus* callus tissues. Plant Physiol 70: 173–178
149. Mok MC, Mok DWS, Marsden KE and Shaw G (1987) The biological activity and metabolism of a novel cytokinin metabolite, O-xylosylzeatin in callus tissue of *P. vulgaris* and *P. lunatus*. Plant Physiol 130: 423–431
150. Mok MC, Mok DWS and Turner JE (1985) Reversed activities of cytokinin bases and ribonucleosides in callus cultures of *Phaseolus lunatus* L. J Plant Physiol 121: 273–280

20 8 23

151. Morris DA (1981) Distribution and metabolism of root-applied cytokinins in *Pisum sativum*. Physiol Plant 52: 251–256
152. Morris JW, Doumas P, Morris OR and Zaerr JB (1990) Cytokinins in vegetative and reproductive buds of *Pseudotsuga menziesii*. Plant Physiol 93: 67–71
153. Motyka V and Kaminek M (1990) Regulation of cytokinin catabolism in tobacco callus cultures. In: Nijkamp HJJ, Van der Plas LHW and Aartrijk J (eds) Progress in Plant Cellular and Molecular Biology, pp 492–497. Dordrecht, The Netherlands: Academic Publishers
154. Murakoshi I, Ikegami F, Ookama N, Haginiwa J and Letham DS (1977) Enzymic synthesis of lupinic acid, a novel metabolite of zeatin in higher plants. Chem Pharm Bull 25: 520–522
155. Nandi SK, Letham DS, Palni LMS, Wong OC and Summons RE (1989) 6-Benzylaminopurine and its glycosides as naturally occurring cytokinins. Plant Sci 61: 189–196
156. Nandi SK, Palni LMS, Letham DS and Wong OC (1989) Identification of cytokinins in primary crown gall tumors of tomato. Plant Cell Environ 12: 273–283
157. Nicholls PB and Murray AW (1968) Adenine phosphoribosyltransferase in plant tissue: some effects of kinetin on enzymic activity. Plant Physiol 43: 645–648
158. Niederwieser JG, Van staden J, Upfold SJ and Drewes FE (1992) Metabolism of 6-benzyladenine by leaf explants of *Lachenalia* during adventitious bud formation. S Afr J Bot 58: 236–238
159. Paces V (1976) Metabolism of cytokinins in barley leaves. Biochem Biophys Res Comm 72: 830–839
160. Paces V and Kaminek M (1976) Effect of ribosylzeatin isomers on the enzymatic degradation of N^6-(Δ^2-isopentenyl) adenosine. Nucleic Acids Res 3: 2309–2314
161. Paces V, Werstiuk E and Hall RH (1971) Conversion of N^6-(Δ^2-isopentenyl) adenosine to adenosine by enzyme activity in tobacco tissue. Plant Physiol 48: 775–778
162. Palmer MV, Horgan R and Wareing PF (1981) Cytokinin metabolism in *Phaseolus vulgaris* L. I: variation in cytokinin levels in leaves of decapitated plants in relation to lateral bud outgrowth. J Exp Bot 32: 1231–1241
163. Palmer MV, Horgan R and Wareing PF (1981) Cytokinin metabolism in *Phaseolus vulgaris* L. Identification of endogenous cytokinins and metabolism of (8-^{14}C)dihydrozeatin in stems of decapitated plants. Planta 153: 297–302
164. Palmer MV, Letham DS and Gunning BES (1984) Cytokinin metabolism in nondividing and auxin-induced dividing explants of *Helianthus tuberosus* L. tuber tissue. Plant Growth Regul 2: 289–298
165. Palmer MV and Palni LMS (1986) Substrate effects on cytokinin metabolism in soybean callus tissue. J Plant Physiol 126: 365–371
166. Palmer MV, Scott IM and Horgan R (1981) Cytokinin metabolism in *Phaseolus vulgaris* L. II. Comparative metabolism of exogenous cytokinins by detached leaves. Plant Sci Lett 22: 187–195
167. Palni LMS, Burch L and Horgan R (1988) The effect of auxin concentration on cytokinin stability and metabolism. Planta 174: 231–234
168. Palni LMS, Palmer MV and Lethan DS (1984) The stability and biological activity of cytokinin metabolites in soybean callus tissue. Planta 160: 242–249
169. Parker CW, Entsch B and Letham DS (1986) Inhibition of two enzymes which metabolise cytokinins. Phytochem 25: 303–310
170. Parker CW and Letham DS (1973) Regulators of cell divisions in plant tissues. XVI. Metabolism of zeatin by radish cotyledons and hypocotyls. Planta 114: 199–218
171. Parker CW and Letham DS (1974) Regulators of cell division in plant tissues. XVIII. Metabolism of zeatin in *Zea mays* seedlings. Planta 115: 337–344
172. Parker CW, Letham DS, Cowley, DE and Macleod JK (1972) Raphanatin, an unusual purine derivative and a metabolite of zeatin. Biochem Biophys Res Commun 49: 460–466
173. Parker CW, Letham DS, Gollnow BI, Summons RE, Duke CC and Macleod JK (1978) Regulators of cell division in plant tissues. XXV. Metabolism of zeatin by lupin seedlings. Planta 142: 239–251
174. Parker CW, Letham DS, Wilson MM, Jenkins ID, Macleod JK and Summons RE (1975) The identity of two new cytokinin metabolites. Ann Bot 39: 375–376
175. Parker CW, Wilson M, Letham DS, Cowley DE and Macleod JK (1973) The glucosylation of cytokinins. Biochem Biophys Res Commun 55: 1370–1376
176. Peters W and Beck E (1992) Endogenous cytokinins in suspension cultured cells of ***Chenopodium rubrum*** at different growth stages. In: Kaminek M, Mok DWS and Zazimalova E (eds) Physiology and Biochemistry of Cytokinins in Plants, pp 71–73. The Hague, The Netherlands: SPB Academic Publishing
177. Pietraface WJ and Blaydes DF (1981) Activity and metabolism of 9-substituted cytokinins during lettuce seed germination. Physiol Plant 53: 249–254
178. Poulton JE and Butt VS (1976) Partial purification and properties of adenosine nucleosidase from elves of spinach beet (*Beta vulgaris* L.). Planta 131: 179–185
179. Ramina A (1979) Aspects of [8-^{14}C]benzylaminopurine metabolism in *Phaseolus vulgaris*. Plant Physiol 63: 298–300
180. Rolle RS and Chism GW (1989) Kinetic comparison of cytokinin nucleosidase activity isolated from normally ripening and mutant tomato varieties. Plant Physiol 91: 148–150
181. Rushing JW (1990) Cytokinins affect respiration, ethylene production, and chlorophyll retention of packaged broccoli florets. HortSci 25: 88–90
182. Schmitz RY, Skoog F, Hecht SM, Bock RM and Leonard NJ (1972) Comparison of cytokinin activities of naturally occurring ribonucleosides and corresponding bases. Phytochem 11: 1603–1610
183. Schmitz RY, Skoog F, Vincze A, Walker GC, Kirkegaard LH and Leonard NJ (1975) Comparison of cytokinin activities of the base, ribonucleoside and 5′-and cyclic-3′,5′-monophosphate ribonucleotides of N^6-isopentenyl-, N^6-benzyl or 8-bromo-adenine. Phytochem 14: 1479–1484
184. Scott IM and Horgan R (1984) Mass-spectrometric quantification of cytokinin nucleotides and glycosides in tobacco crown-gall tissue. Planta 161: 345–354
185. Scott IM, Martin GC, Horgan R and Heald JK (1982) Mass spectrometric measurement of zeatin glycoside levels in *Vinca rosea* L. crown gall tissue. Planta 154: 273–276
186. Scott IM, McGaw BA, Horgan R and Williams PE (1982) Biochemical studies on cytokinins in *Vinca rosea* crown gall tissue. In: Wareing PF (ed) Plant Growth Substances 1982, pp 165–174. London, UK: Press
187. Scott RA and Liverman JL (1956) Promotion of leaf expansion by kinetin and benzylaminopurine. Plant Physiol 31: 321–322
188. Senesi S, Falcone G, Mura U, Scarrella F and Ipata PL (1976) A specific adenosine phosphorylase, distinct from purine

nucleoside phosphorylase. Fed Eur Biochem Soc 64. 353–356

189. Shaw G, Smallwood BM and Steward FC (1968) Synthesis and cytokinin activity of the 3-, 7-, and 9-methyl derivatives of zeatin. Experientia 24: 1089–1090
190. Singh S, Palni LMS and Letham DS (1992) Cytokinin biochemistry in relation to leaf senescence. V Endogenous cytokinin levels and metabolism of zeatin riboside in leaf discs from green and senescent tobacco (*Nicotiana rustica*) leaves. J Plant Physiol 139: 279–283
191. Skoog F and Armstrong DJ (1970) Cytokinins. Annu Rev Plant Physiol 21: 359–384
192. Skoog F, Hamzi HQ, Szweykowska AM, Leonard NJ, Carraway KC, Fujii T, Helgeson JP and Loeppky RN (1967) Cytokinins: structure/activity relationships. Phytochem 6: 1169–1192
193. Skoog F and Schmitz RY (1979) Biochemistry and physiology of cytokinins. In: Lutwack F (ed) Biochemical Actions of Hormones. Volume VI, pp 335–355. New York, USA: Academic Press
194. Smith AR and Van Staden J (1978) Changes in endogenous cytokinin levels in kernels of *Zea mays* L. during imbibition and germination. J Exp Bot 29: 1067–1075
195. Sondheimer E and Tzou D-S (1971) The metabolism of hormones during seed germination and dormancy. II. the metabolism of 8-^{14}C zeatin in bean axes. Plant Physiol 47: 516–520
196. Spiess LD (1975) Comparative activity of isomers of zeatin and ribosylzeatin on *Funaria hygrometrica*. Plant Physiol 55: 583–585
197. Strnad M, Peters W, Beck E and Kaminek M (1992) Immunodetection and identification of N^6-(*o*-hydroxybenzylamino)purine as a naturally occurring cytokinin in *Populus* × *canadensis* Moench cv. Robusta leaves. Plant Physiol 99: 74–80
198. Summons RE, Entsch B, Letham DS, Gollnow BI and Macleod JK (1980) Regulators of cell division in plant tissues. XXVIII. Metabolites of zeatin in sweet-corn kernels: purification and identification using high performance liquid chromatography and chemical ionisation mass spectrometry. Planta 147: 422–434
199. Szweykowska A, Gwozdz E and Spychala M (1981) The cytokinin control of protein synthesis in plants. In: Guern J and Peaud-Lenoël D (eds) Metabolism and Molecular Activities of Cytokinins, pp 211–217. Berlin, Germany: Springer-Verlag
200. Tao G-Q, Letham DS, Hocart CH and Summons RE (1991) Inhibitors of cytokinin metabolism. III. The inhibition of cytokinin N-glucosylation in radish cotyledons. Plant Growth Regul 10: 179–185
201. Taya Y, Tanaka Y and Nishimura S (1978) 5^1-AMP is a direct precursor of cytokinin in *Dictyostelium discoideum*. Nature 271: 545–547
202. Taylor JS, Koshioka M, Pharis RP and Sweet GB (1984) Changes in cytokinin and gibberellin-like substances in *Pinus radiata* buds during lateral shoot initiation and the characterisation of ribosyl zeatin and a novel ribosyl zeatin glycoside. Plant Physiol 74: 626–631
203. Ternine C and Laloue M (1980) Kinetics of N^6-(Δ^2-isopentenyl)adenosine degradation in tobacco cells. Evidence of a regulatory mechanism under the control of cytokinins. Plant Physiol 65: 1090–1095
204. Thomas TH and Blakesley D (1987) Practical and potential uses of cytokinins in agriculture and horticulture. Brit Plant Growth Regul Group Mono 14: 69–83
205. Turner JE, Mok DWS, Mok MC and Shaw G (1987) Isolation and partial purification of the enzyme catalysing the formation of novel zeatin metabolites in *Phaseolus vulgaris* embryos. Proc Natl Acad Sci USA 84: 3714–3717
206. Tzou D-S, Galston EC and Sondheimer E (1973) The metabolism of hormones during seed germination and release from dormancy. III. The effects and metabolism of zeatin in dormant and nondormant ash embryos. Plant Physiol 51: 894–897
207. Van der Krieken WM, Croes AF, Barendse GWM and Wullems GJ (1988) Uptake and metabolism of benzyladenine in the early stage of flower bud development *in vitro* in tobacco. Physiol Plant 74: 113–118
208. Van Staden J (1973) Changes in endogenous cytokinins of lettuce seed during germination. Physiol Plant 28: 222–227
209. Van Staden J (1973) A comparison of the effect of 6-benzylaminopurine derivatives on some aspects of plant growth. Physiol Plant 29: 137–139
210. Van Staden J (1976) Seasonal changes in the cytokinin content of *Ginkgo biloba* leaves. Physiol Plant 38: 1–5
211. Van Staden J (1976) The identification of zeatin glucoside from coconut milk. Physiol Plant 36: 123–126
212. Van Staden J (1979) The metabolism of zeatin by cell-free plant extracts. Z Pflanzenphysiol 92: 399–405
213. Van Staden J (1983) Short term metabolism of different concentrations of zeatin applied to soybean callus. Z Pflanzenphysiol 109: 163–169
214. Van Staden J (1983a) Long term metabolism of zeatin supplied to soybean callus. Z Pflanzenphysiol 111: 423–428
215. Van Staden J and Bayley AD (1991) Fate of benzyladenine metabolites extracted from tomato shoots in biological systems. Plant Growth Regul 10: 117–124
216. Van Staden J, Bayley AD, Upfold SJ and Drewes FE (1990) Cytokinins in cut carnation flowers. VIII. Uptake, transport and metabolism of benzyladenine and the effect of benzyladenine derivatives on flower longevity. J Plant Physiol 135: 703–707
217. Van Staden J and Davey JE (1981) Cytokinin activity in *Lupinus albus*. VI. Translocation and metabolism of (8-^{14}C)zeatin applied to the leaves and fruits of fruiting plants. Physiol Plant 51: 49–52
218. Van Staden J and Dimalla GG (1978) Endogenous cytokinins and the breaking of dormancy and apical dominance in potato tubers. J Exp Bot 29: 1077–1084
219. Van Staden J and Drewes FE (1991) The biological activity of cytokinin derivatives in the soybean callus bioassay. Plant Growth Regul 10: 109–115
220. Van Staden J and Drewes FE (1992) The stability and metabolism of benzyladenine glucosides in soybean callus. J Plant Physiol 140: 92–95
221. Van Staden J and Drewes FE (1992a) Metabolism of ribosylzeatin-5^1-monophosphate by soybean callus. Plant Growth Regul 11: 189–191
222. Van Staden J and Hutton MJ (1982) Metabolism of 8[^{14}C]*t*-zeatin in soybean callus. Z Pflanzenphysiol 106: 355–365
223. Van Staden J and Mallett JA (1988) Metabolism and transport of [8-^{14}C]benzyladenine applied to rootless shoots and the roots of intact tomato plants. Plant Physiol Biochem 26: 47–54

22 & 23

224. Van Staden J and Mooney PA (1987) The effect of IAA on the metabolism of kinetin by *Catharanthus roseus* crown-gall callus. J Plant Physiol 131: 297–303
225. Van Staden J and Papaphilippou AP (1977) Biological activity of O-β-D-glucopyranosylzeatin. Plant Physiol 60: 649–650
226. Venis M (1985) Hormone Binding Sites in Plants. London, UK: Longman Press
227. Vogelmann TC, Bornman CH and Nissen P (1984) Uptake of benzyladenine in explants of *Picea abies* and *Pinus sylvestris*. Physiol Plant 61: 513–517
228. Vonk CR and Davelaar E (1981 8-^{14}C-Zeatin metabolites and their transport from leaf to phloem exudate of *Yucca*. Physiol Plant 52: 101–107
229. Wagner BM and Beck E (1993) Cytokinins in the perennial herb *Urtica dioica* L. as influenced by its nitrogen status. Planta 190: 511–518
230. Wang TL, Thompson AG and Horgan R (1977) A cytokinin glucoside from the leaves of *Phaseolus vulgaris* L. Planta 135: 285–288
231. Wang TP (1955) Hydrolytic nucleosidases. Methods Enzymol 2: 456–464
232. Wareing PF, Horgan R, Henson IE and Davis W (1977) Cytokinin relations in the whole plant. In: Pilet PE (ed) Plant Growth Regulation, pp 147–153. Berlin, Germany: Springer-Verlag
233. Whitacker BD and Kende H (1974) Bud formation in *Funaria hygrometrica*. A comparison of the activities of three cytokinins with their ribosides. Planta 121: 93–96
234. Whitty CD and Hall RH (1974) A cytokinin oxidase in *Zea mays*. Can J Biochem 52: 789–799
235. Wilson MM, Gordon ME, Letham DS and Parker CW (1974) Regulators of cell division in plant tissues (XIX). The metabolism of 6-benzylaminopurine in radish cotyledons and seedlings. J Exp Bot 25: 725–732
236. Woolley DJ and Wareing PF (1972) The role of roots, cytokinins and apical dominance in the control of lateral shoot form in *Solanum andigena*. Planta 105: 33–42
237. Yoshida R and Oritani T (1972) Cytokinin glucoside in roots of the rice plant. Plant Cell Physiol 13: 337–343
238. Zhang R, Hocart CH and Letham DS (1989) Inhibitors of cytokinin metabolism. II. Inhibition of cytokinin-alanine conjugation in soybean leaves and associated effects on senescence. Plant Growth Regul 8: 327–330
239. Zhang R and Letham DS (1989) Cytokinin biochemistry in relation to leaf senescence. III. The senescence retarding activity and metabolism of 9-substituted 6-benzylaminopurines in soybean leaves. Plant Growth Regul 8: 181–197
240. Zhang R, Letham DS, Parker CW, Schroeder H and Higgins TJV (1987) Retardation of soybean leaf senescence and associated effects on seed composition. Plant Growth Regul 6: 15–21
241. Zhang R, Letham DS, Wong OC, Noodén LD and Parker CW (1987) The metabolism of 6-benzylaminopurine in soybean leaves and the inhibition of its conjugation. Plant Physiol 83: 334–340
242. Zimmerman TP, Gersten NB, Ross AF and Miech RP (1971) Adenine as a substrate for purine nucleoside phosphorylase. Can J Biochem 49: 1050–1054